Maternal brain involvement in (pre)eclampsia

Pathophysiology and long-term consequences

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1

INTRODUCTION

Preeclampsia and eclampsia

Preeclampsia is a pregnancy specific disorder which complicates about 5 to 7% of all pregnancies and is characterized by the presence of hypertension (blood pressure systolic ≥ 140 mmHg and/or diastolic ≥ 90 mmHg) and proteinuria after the 20th week of pregnancy in a previously normotensive woman.[1] Although the pathophysiology of preeclampsia has been the focus of many studies, the exact mechanism underlying the disease remains unclear. Currently leading hypotheses concern poor placentation followed by oxidative stress and release of substances from the intervillous space into the maternal circulation such as soluble endoglin, soluble fms-like tyrosine kinase-1 (sFlt-1) and syncytiotrophoblast microparticles. These substances may subsequently lead to a maternal inflammatory response and generalized endothelial dysfunction resulting in a decreased circulating volume and altered vascular reactivity.[1]

Preeclampsia is a systemic disorder that can affect several maternal organs, including the kidneys, liver and brain. Involvement of the brain is most evident in case of eclampsia which is characterized by tonic-clonic convulsions or coma, and may develop before, during or after labor.[2, 3] Several other neurological symptoms can be seen including severe and persistent headache, nausea, vomiting, altered mental status and photophobia.[4, 5] In addition, visual disturbances may accompany (pre)eclampsia and are reviewed in **Chapter 6**.

Eclampsia is a relatively rare but serious complication of preeclampsia, and largely contributes to preeclampsia-related maternal and neonatal mortality and morbidity worldwide. In the Netherlands, eclampsia occurs in 6.2 per 10,000 deliveries and is responsible for death in 1 out of 74 eclamptic cases (1.4%).[6] In the period 1993-2005, the overall Dutch maternal mortality ratio was 12.1 per 100,000 live births. The preeclampsia-related mortality ratio was 3.5 per 100,000, which makes preeclampsia the leading cause of maternal mortality in the Netherlands. Of all preeclampsia-related deaths, 61% is due to cerebral complications.[7] In other developed countries, incidence rates of eclampsia vary from 2.4 to 8.0 per 10,000 deliveries[8-11] and case fatality rates range from 0.5 to 1.8 %.[3, 10] Unfortunately, eclampsia is more common in developing countries[12] with incidence rates of 29 to 220 per 10,000 deliveries and maternal outcome is worse with case fatality rates of 5.0 to 26.5 %.[13-16]

Animal models of preeclampsia

Both preeclampsia and eclampsia spontaneously occur only in the human and in primates.[17] During the last decades, much effort has been put into the development of animal models for preeclampsia. Ideally, such a model should exhibit the full spectrum of biochemical and clinical features of human preeclampsia. However, so far no model has been able to reproduce all preeclampsia aspects, but several models have been identified to mimic at

least part of the syndrome. These models in several species include reduced uteroplacental perfusion by clipping the aorta or uterine arteries, chronic nitric oxide synthase inhibition, adriamycin-induced nephropathy, insulin resistance, renin angiotensin or sympathetic nervous system overactivity, antagonism of angiogenic factors by sFlt-1 and inflammatory models with low-dose endotoxin or tumor necrosis factor α administration.[17]

In **Chapter 4** and **5**, the low-dose endotoxin-infused pregnant rat was used as a model for preeclampsia to study the effects of this disease on the brain. Pregnant rats in this model become hypertensive after low-dose endotoxin infusion through a jugular vein cannula at day 14 of pregnancy.[18] Furthermore, these rats have been shown to exhibit proteinuria, disseminated intravascular coagulation with decreased platelet numbers and endothelial cell activation,[18-21] which are all features of human preeclampsia. In addition, the syndrome is pregnancy-specific since nonpregnant rats treated with endotoxin remain asymptomatic.[18, 19] The underlying mechanism of the development of the preeclamptic-like syndrome in this model is considered to be a generalized low-grade inflammatory response induced by endotoxin,[19, 22] which is similar to the systemic intravascular inflammatory state observed in preeclamptic patients.[1] Additionally, oxidative stress may have a role as well, which seems also the case in human preeclampsia.[23]

Pathophysiology of eclampsia

The pathophysiology of eclampsia has not been completely elucidated. Nowadays, two opposing theories exist about the mechanism underlying the occurrence of eclampsia, both concerning failure of autoregulation of cerebral blood flow.

The first theory proposes that eclampsia arises from cerebral ischemia. In this theory, acute hypertension leads to vasospasm due to cerebral "overautoregulation". As a result, cerebral hypoperfusion may induce ischemia, cytotoxic edema formation and eventually cerebral infarction. Evidence for this theory comes from catheter angiography and MR angiography demonstrating focal or diffuse vasoconstriction of the cerebral vasculature.[24-26]

The second theory entails loss of cerebral autoregulation, in which, in the presence of some degree of endothelial dysfunction, an increase in blood pressure is thought to exceed the upper autoregulatory limit. This may cause forced dilatation of the cerebral vasculature leading to cerebral hyperperfusion, blood-brain barrier disruption and cerebral vasogenic edema formation.[27-29] This edema is considered to be the underlying cause of the neurological symptoms in eclampsia and resolves upon recovery of endothelial cell dysfunction and lowering of blood pressure to values within the autoregulatory limits.[30-32]

Since the emergence of advanced cranial imaging techniques, it has become apparent that cerebral edema in eclampsia is consistent with vasogenic edema.[33-35] Therefore, the first theory which held ischemia as the sole mechanism underlying eclampsia seems unlikely. In fact, the second theory of loss of cerebral autoregulation is currently the most

popular.

In 1996, Hinchey et al described a syndrome in a series of patients which all had neuroimaging findings consistent with cerebral edema, mainly in the posterior regions of the brain.[30] Furthermore, these patients demonstrated similar neurological symptoms, including headache, visual disturbances, altered mental status and seizures. This syndrome, named 'reversible posterior leukoencephalopathy syndrome', was associated with a variety of disorders, one of which was eclampsia. Since that time, eclampsia is considered to be a form of this syndrome, which is nowadays mostly referred to as 'posterior reversible encephalopathy syndrome' (PRES).[36, 37]

Posterior Reversible Encephalopathy Syndrome

PRES is a clinicoradiological entity, which is associated with a wide range of clinical problems in both the adult as well as the pediatric population.[38] PRES has most commonly been reported in patients with eclampsia, immunosuppression after organ transplantation, and severe hypertension but is also associated with renal inflammatory conditions, chemotherapy and several autoimmune diseases.[30, 38, 39]

PRES can present with various neurological symptoms, of which headache, nausea and vomiting, seizure, altered mental state and visual disturbances such as blurred vision and cortical blindness are most common.[30] As its name suggests, the classical radiologic imaging pattern in PRES consists of bilateral edema predominantly in the posterior portion of the cerebral hemispheres, especially the parieto-occipital regions. However, frontal and temporal lobe, cerebellum, basal ganglia and brainstem involvement is also seen in PRES patients.[30, 38-40] While the subcortical white matter is mainly affected by edema, gray matter involvement and hemorrhage may also occur.[39, 41] Although the edema is often apparent on computed tomography (CT) imaging as hypointense lesions, the edema is best depicted by magnetic resonance imaging (MRI). On MRI, PRES lesions appear as hypointensities on T1 sequence and hyperintensities on T2 and fluid attenuation inversion recovery (FLAIR) sequences.[30, 38] Using diffusion weighted imaging (DWI), these areas have normal or decreased signal intensity while they show increased apparent diffusion coefficients (ADC).[33, 35] This MRI imaging pattern of PRES is consistent with vasogenic edema. However, in some cases DWI demonstrate small areas of cytotoxic edema within areas of vasogenic edema, which has been suggested to result from extensive vasogenic edema leading to decreased cerebral perfusion, impaired cerebral blood flow and ischemia.[35, 39, 42]

The exact mechanism leading to cerebral edema in PRES is not completely understood. While acute hypertension is considered to play a central role in the pathophysiology of PRES, evident hypertension is not always present.[43] In fact, 20-30% of patients developing PRES are normotensive or have hypertensive values which do not exceed the generally accepted upper limit of cerebral autoregulation,[43-45] which is the capacity of the cerebral vasculature to keep cerebral blood flow relatively constant in the presence of changes in

cerebral perfusion pressure (see below). Interestingly, in pathological conditions associated with PRES a certain degree of endothelial dysfunction is usually present.[43] While the role of endothelial dysfunction in the development of PRES has not been elucidated, it may influence cerebral autoregulation and the blood-brain barrier.

The cerebral vasculature seems to play a crucial role in the pathophysiology of PRES, and eclampsia in particular. In this context, several aspects of the cerebral circulation are of interest and will be discussed in the following paragraphs, including cerebral autoregulation and the blood-brain barrier.

Cerebral autoregulation

Under normal conditions, cerebral blood flow is maintained at a relatively constant level despite changes in cerebral perfusion pressure. The ability of the brain to maintain this relatively constant blood flow during changes in perfusion pressure is called autoregulation and is brought about by changes in arterial diameter and cerebrovascular resistance (Figure 1).[46] Between arterial pressures of about 50-60 to 150-160 mmHg, cerebral blood flow is kept constant and is called the autoregulatory plateau.[47] However, in case of blood pressures below or above these limits, autoregulation may be lost.[46] During acute hypertension, as in eclampsia, the upper limit of cerebral autoregulation is exceeded and the myogenic tone of cerebral arteries and arterioles is overcome due to excessive intravascular pressure.[46,48] In this state of forced dilatation, cerebral blood flow increases linearly with increases in blood pressure.[48, 49] Consequently, the blood-brain barrier can be disrupted due to excessive hydrostatic pressure leading to vasogenic edema formation.[29, 50, 51] When blood pressure is reduced to values within the autoregulatory range, cerebral blood flow normalizes and edema is thought to resolve.[30, 52]

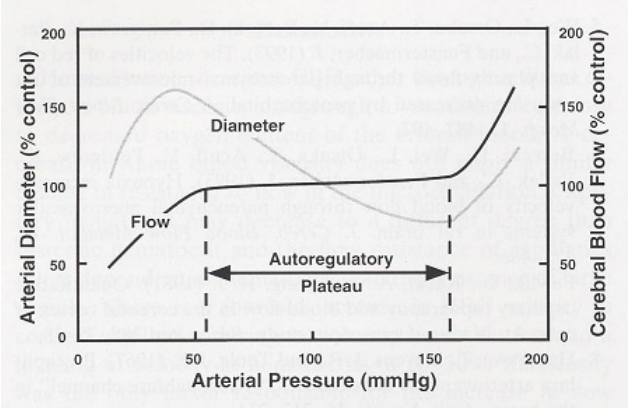

Figure 1. The cerebral autoregulation curve. Adapted from Chillion and Baumbach 1997 [47] and used with permission from Elsevier Limited.

Several mechanisms have been proposed to cause changes in cerebral artery diameter in order to maintain a constant cerebral blood flow, including neurogenic, metabolic and myogenic mechanisms.[53, 54] Concerning the neurogenic mechanism, neuronal innervation of the cerebral vasculature may control cerebral blood flow and comprises extrinsic innervation arising from the peripheral nervous system, and intrinsic innervation originating from within the brain. Although under resting conditions extrinsic sympathetic and trigeminal innervation appears to have little effect on cerebral blood flow, a role for extrinsic parasympathetic and intrinsic innervation has been suggested.[46, 55-57] The metabolic mechanism is thought to be flow-dependent: reduction of cerebral blood flow results in accumulation of metabolic substances that cause vasodilatation.[53] This way, higher metabolic demand leads to increased cerebral blood flow. In contrast, the myogenic control mechanism suggests a pressure-dependent mechanism by which vascular wall smooth muscle cells respond to changes in transmural pressure.[46, 58] This intrinsic response induces vascular constriction when transmural pressure increases and relaxation during pressure decreases. In addition to these three mechanisms underlying cerebral autoregulation, endothelial-derived factors are also thought to influence cerebral autoregulation by exerting relaxing or constrictive influences on vascular smooth muscle cells. Release of these factors can be induced by various stimuli, including shear stress, hormones and neuronal signals.[59]

Chronic hypertension influences cerebral autoregulation by inducing remodeling and hypertrophy of the cerebral arteries and arterioles, thereby normalizing wall stress by increasing wall thickness and reducing vascular diameter.[60, 61] These structural changes of the vascular wall are considered to be protective mechanisms for the cerebral circulation by inducing a rightward shift of the cerebral autoregulation curve.[62] This way, the pressure at which loss of cerebral autoregulation occurs increases to higher values, i.e., the cerebral blood flow autoregulatory curve shifts to the right.

Since impaired cerebral autoregulation seems to have a crucial role in the pathophysiology of PRES/eclampsia, a number of studies have focused on the influence of pregnancy per se on cerebral autoregulation. A considerable number of eclamptic women never reach blood pressures that exceed the upper limit of autoregulation.[3, 10] Therefore, it has been suggested that pregnancy may shift the upper limit of the autoregulation curve to the left, thereby leading to breakthrough of autoregulation and hyperperfusion at lower blood pressure compared to nonpregnant subjects. In support of this hypothesis, Cipolla et al found that posterior cerebral arteries from late-pregnant rats in vitro exhibit less myogenic reactivity and undergo forced dilatation at lower pressures compared to nonpregnant animals.[63, 64] However, in contrast to these findings, pressure of forced dilatation did not differ between late-pregnant and nonpregnant animals in vivo.[65] Therefore, whether there is an actual pregnancy-induced leftward shift of the cerebral autoregulation curve remains to be elucidated.

Although studies in normal pregnancy may certainly add to our understanding of the cerebrovascular pathophysiology of preeclampsia, the influence of preeclampsia on the cerebral circulation is of particular interest. Studies addressing structure and function of cerebral arteries in rat models of pregnancy hypertension are limited. In Dahl salt-sensitive rats, that become hypertensive due to a high-salt diet, myogenic reactivity was diminished during pregnancy, regardless of presence of hypertension.[64] In addition, while hypertension in nonpregnant rats causes inward remodeling and medial hypertrophy of posterior cerebral arteries, arteries from late-pregnant rats lacked this response.[64] This lack of hypertensive remodeling was also shown in a study using nitric oxide synthase inhibition to raise blood pressure in nonpregnant and late-pregnant rats.[66] Both diminished myogenic reactivity and lack of hypertensive remodeling may predispose the brain to breakthrough of autoregulation when blood pressure is increased, as observed in eclampsia. How preeclampsia, induced by low-dose endotoxin infusion in pregnant rats, affects the function and structure of the cerebral circulation has not been elucidated and was investigated in **Chapter 4**.

Blood-brain barrier and vasogenic edema

Another unique feature of the cerebral circulation is the presence of the blood-brain barrier (BBB). This BBB is formed by the cerebral endothelium and distinguishes the brain from other tissues pertaining to the processes of extravasation of water and solutes from the blood stream. In other tissues, plasma proteins are kept within the capillaries but water and solutes can freely move between the vascular endothelial cells into the surrounding tissue.[67] In the brain, however, the BBB prevents extravasation of both small and large solutes including ions and plasma proteins.[67] This is established by the presence of high electrical resistance tight junctions between the endothelial cells which prevent paracellular flux.[68, 69] In addition, cerebral endothelial cells lack fenestrations and have a low rate of pinocytosis, which both restrict transcellular transport.[68, 70] In this way, the BBB protects the brain against formation of vasogenic edema since water movement due to hydrostatic pressure in the capillaries is immediately opposed by the osmotic pull of both plasma proteins and ions.[67]

It is of importance to understand the influence of pregnancy on the BBB since the vasogenic edema seen in eclamptic patients is considered to be a consequence of BBB disruption.[71] Apparently, during acutely increased hydrostatic pressure, the BBB can be disrupted with resultant vasogenic edema formation.[71, 72] An in vivo study in nonpregnant and late-pregnant rats with acute hypertension showed that while pregnancy did not affect the pressure at which breakthrough of cerebral autoregulation occurred, only the late-pregnant animals developed significant brain edema in response to this autoregulatory breakthrough.[65] These results suggest that pregnancy predisposes to cerebral edema formation during acute hypertension. In **Chapter 2**, we aimed to provide insight into the mechanism behind this vulnerability. Since increases in BBB permeability may promote

greater edema formation in response to increased hydrostatic pressure, we investigated the effect of pregnancy on hydraulic conductivity (permeability to water in response to hydrostatic pressure) and endothelial cell permeability to Lucifer Yellow of rat posterior cerebral arteries. Furthermore, we assessed whether estrogen has a role in the effects of pregnancy on BBB permeability.

Aquaporins

Also aquaporins (AQPs) have been proposed to have a role in brain water homeostasis and cerebral edema formation and resolution.[73] AQPs are a family of channel forming transmembrane proteins consisting of 13 members, AQP0 through AQP12.[73] These proteins all facilitate bidirectional movement of water through cell membranes in a variety of cell types.[74, 75] Some AQPs are also permeable to glycerol, urea or other solutes.[73] Currently, seven of the AQP family members have been identified in the rodent brain, of which three are well described in vivo, namely AQP1, AQP4, and AQP9.[73]

AQP1 is permeable only to water and has been localized within epithelial cells of the choroid plexus.[76, 77] Given its location and the finding that AQP1-deficient mice have reduced cerebrospinal fluid (CSF) formation, AQP1 is proposed to have a role in CSF formation.[78] AQP4 is the most abundant AQP in the brain, and its permeability is restricted to water.[73] Expression of AQP4 is found in several brain regions including the cortex, hippocampus, magnocellular hypothalamic nuclei, cerebellum and brain stem.[75, 79] In these regions AQP4 is expressed in astrocytic endfeet bordering the ventricles, subarachnoid space, and the blood vessels.[80] Some studies have also demonstrated endothelial AQP4 expression,[81, 82] however, others did not.[80, 83] Given its location at the blood-brain and brain-CSF interfaces, AQP4 is proposed to have a role in brain water homeostasis. Furthermore, colocalization with the potassium channel $K_{ir}4.1$ suggests involvement of APQ4 in potassium homeostasis.[84] AQP9 facilitates diffusion of both water and several solutes such as glycerol, urea and monocarboxylates.[85] Expression of AQP9 is found in glia and catecholaminergic neurons.[86, 87] One study also showed AQP9 in endothelial cells of pial vessels.[87] The localization of AQP9 suggests involvement in cerebral water homeostasis. In addition, a role in brain energy metabolism by facilitating diffusion of glycerol and monocarboxylates has been proposed.[87]

Since cerebral edema formation is basically an expression of loss of water homeostasis in the brain, AQPs have been proposed to play a role in cerebral edema formation. In fact, several studies in different animal models have focused on the role of AQP1, AQP4 and AQP9 in cerebral edema formation and resolution. For example, in a rodent model of subarachnoid hemorrhage, which is accompanied by cerebral edema formation, AQP1 expression appears increased.[88] In a mouse model of transient focal cerebral ischemia, also accompanied by cerebral edema, AQP9 is upregulated in astrocytes around the infarct border.[89] However, this upregulation was not correlated with brain swelling, suggesting a

lack of an important role for AQP9 in water movement associated with vasogenic edema formation in ischemic brain injury.[90]

Of the cerebral AQPs, AQP4 has been most extensively studied in relation to cerebral edema. Its role in cerebral edema formation was first described when AQP4 knockout mice were found to have reduced cytotoxic edema after ischemic stroke and acute hyponatremia.[91] Later studies have confirmed this concept, with altered AQP4 expression in animal models of trauma and ischemia as well as in human subarachnoid hemorrhage and brain tumour, all conditions that are associated with cerebral edema.[90, 92-97] In addition to its role in edema formation, a contribution of AQP4 in edema resolution has been proposed. In studies using a freeze injury model of vasogenic edema, AQP4 knockout mice exhibited increased brain water content compared to wild type mice.[98, 99]

Since cerebral AQP expression may have a role in cerebral edema during eclampsia, the regional expression of AQP1, 4 and 9 in the brain during pregnancy was evaluated in **Chapter 3**.

Biomarkers of brain injury

Once preeclamptic patients start to seize, the presence of central nervous system (CNS) involvement is clear. However, this is not always the case in the period preceding the seizures. Since these difficulties in diagnosing the presence of CNS involvement are faced in several patient categories, identification of non-invasive biomarkers of neuronal and BBB injury has become of interest. Ideally, these biomarkers are brain specific substances released into cerebrospinal fluid and/or peripheral blood in which elevated levels can be measured during the manifestation of CNS disorders. Several candidate biomarkers have been proposed, including neuron-specific enolase (NSE), glial fibrillary acidic protein (GFAP) and S100B.[100,101]

S100B is member of a family of acidic calcium-binding proteins and is mainly expressed in astrocytes and Schwann cells within the CNS.[102] Outside the CNS, S100B is expressed in a variety of cell types including melanocytes, chondrocytes and Langerhans cells, however, approximately 95% of S100B is located in the CNS.[102-104] Therefore, several studies have related increased peripheral S100B levels and S100B brain expression to the process of neuroinflammation, a key component of several CNS disorders involved in both neural tissue damage and regeneration.[101, 104-107] While the exact mechanism by which such level or expression increases has not been completely elucidated, proposed mechanisms include astrocytic death, reactive gliosis and BBB dysfunction.[104, 105, 107-110]

In preeclampsia, there may also be a place for use of neuroinflammatory biomarker levels, including S100B, in the diagnosis of (impending) cerebral involvement. In addition to early recognition, cerebrovascular complications might eventually even be prevented when cerebral involvement in preeclampsia is diagnosed at an early stage. However, knowledge about S100B as a neuroinflammatory biomarker in pregnancy and (pre)eclampsia is

scarce.[104] In contrast, many studies have assessed S100B in various neuropathological states in nonpregnant subjects. For instance, elevated S100B serum levels were observed in both animal and human studies of cerebral hemorrhage, ischemic stroke, traumatic injury and iatrogenic BBB disruption.[102, 105-107, 111] In addition, increased S100B brain expression was demonstrated in stroke-prone spontaneously hypertensive rats,[112] chronic cerebral hypoperfusion in rats[108] and septic encephalopathy in pigs.[113]

In **Chapter 5**, the presence and extent of brain injury was assessed in the low-dose endotoxin-treated pregnant rat, a preeclampsia animal model, using the neuroinflammatory marker S100B.

Long-term consequences of eclampsia

Eclampsia and/or PRES have long been held to be reversible disorders, of which patients could expect to recover completely. However, during the last decade doubts have been raised concerning this full recovery. Approximately 25% of formerly eclamptic women appeared to have persistent white matter lesions (WML) consistent with tissue loss or gliosis on cerebral MRI several weeks postpartum.[33, 35] In addition, on long-term MRI follow-up formerly eclamptic women had significantly more WML compared to healthy parous controls.[114] Two theories concerning the etiology of these lesions following eclampsia have been proposed. The first theory suggests a causal relationship between WML and PRES in which vasogenic edema progresses to such an extent that regional cerebral perfusion pressure decreases and blood flow diminishes to ischemic levels leading to areas of cytotoxic edema and infarction.[42] On the other hand, such WML have been suggested to develop independently of pregnancy as an early expression of increased susceptibility for cerebro-/cardiovascular disease in these women. Epidemiological studies have shown an increased risk for cerebro-/cardiovascular disease later in life in women with a history of preeclampsia, including risk for hypertension, ischemic heart disease, stroke and venous thromboembolism.[115] While the mechanism behind this association remains to be unraveled, preeclampsia and atherosclerosis have several risk factors in common.[116, 117] Therefore, preeclampsia and WML might both simultaneously develop because of the predisposition to cerebro-/cardiovascular disease in these women.

The clinical implications of WML in formerly eclamptic women are largely unknown. In the elderly population, WML have been related to cognitive decline and dementia.[118, 119] In a follow-up study, a substantial part of formerly eclamptic women report several neurocognitive limitations months to years after eclampsia, including problems with concentrating and recalling.[120] These results are in line with a study by Aukes et al demonstrating impaired subjective cognitive functioning years following eclampsia.[121] In addition to subjective cognitive impairment, visual disturbances are reported by a significant number of the formerly eclamptic women.[120] While most women recover from

the visual disturbances seen during the acute phase of eclampsia, incidental permanent visual field loss due to cerebral infarction has been described after both obstetric and non-obstetric PRES.[122, 123] This raised the question whether WML in formerly eclamptic women may cause visual field loss, and is subject of **Chapter 7**. To gain further insight into the possible consequences and etiology of WML after eclampsia, evaluation of the location of these lesions may be elucidating, and is therefore assessed in **Chapter 8**.

Aims of thesis

Part I – Animal studies

- To investigate the underlying mechanism by which pregnancy increases blood-brain barrier permeability. (**Chapter 2**)
- To determine the regional distribution of aquaporin 1, 4 and 9 in brain and possible pregnancy-related changes in distribution. (**Chapter 3**)
- To assess how preeclampsia influences the function and structure of cerebral resistance arteries and to investigate whether peripheral resistance arteries are similarly affected. (**Chapter 4**)
- To assess the presence and extent of brain injury during preeclampsia using a neuroinflammatory biomarker. (**Chapter 5**)

Part II – Human studies

- To review the visual disturbances that can be encountered in patients with (pre) eclampsia. (**Chapter 6**)
- To assess whether cerebral white matter lesions in formerly eclamptic women influence visual functioning in the long term. (**Chapter 7**)
- To provide insight into the regional distribution of cerebral white matter lesions following preeclampsia and eclampsia. (**Chapter 8**)

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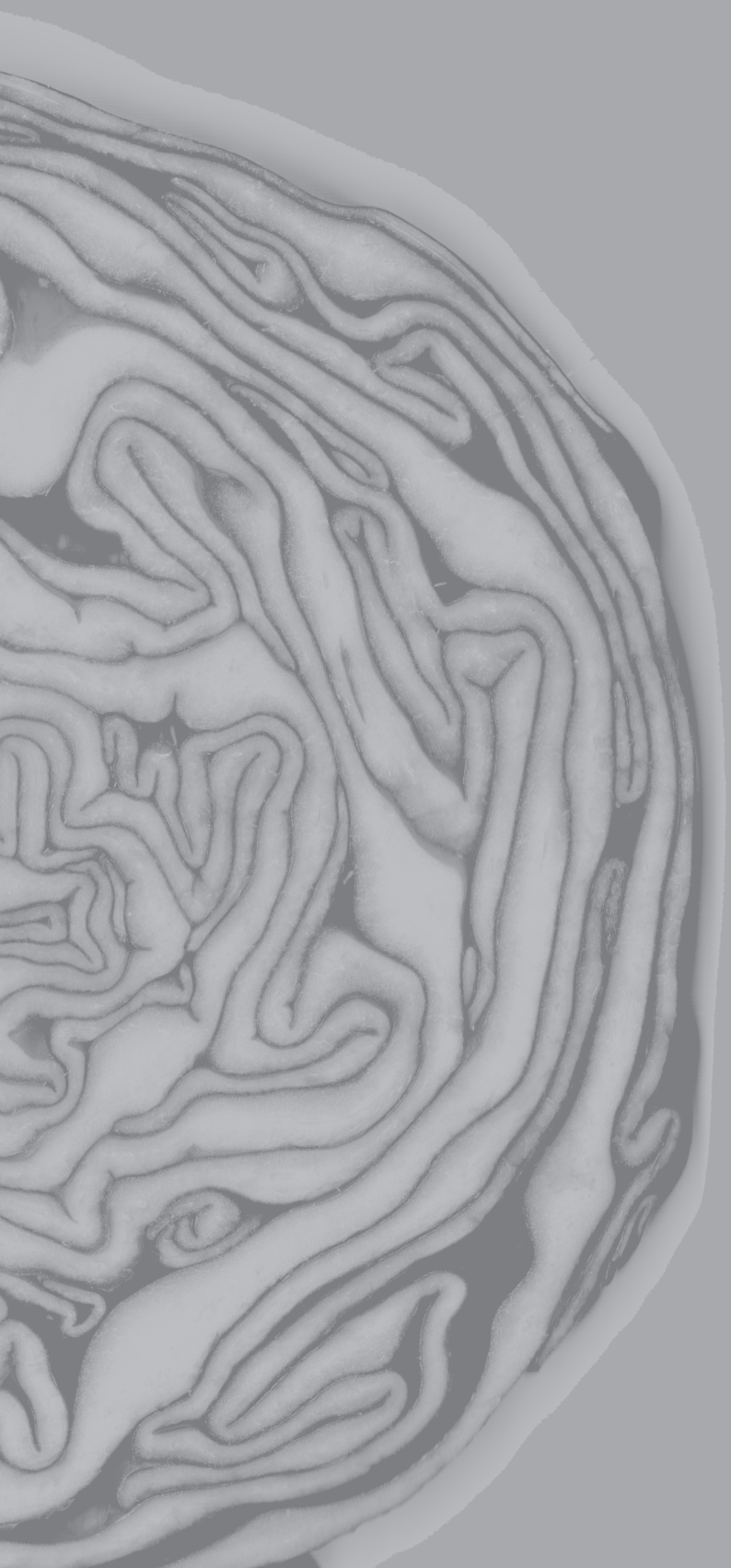

PART 1

ANIMAL STUDIES

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2

THE EFFECT OF PREGNANCY AND ESTROGEN ON BLOOD-BRAIN BARRIER PERMEABILITY IN RESPONSE TO ELEVATED PRESSURE

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Abstract

Eclampsia is similar to posterior reversible encephalopathy syndrome in which acutely elevated blood pressure causes autoregulatory breakthrough, blood-brain barrier (BBB) disruption, and edema formation. We previously showed that late-pregnant (LP) animals developed cerebral edema during breakthrough, a response that was absent in nonpregnant (NP) animals. The current study hypothesized that pregnancy predisposes to cerebral edema during acute hypertension by enhanced cerebral endothelial cell permeability in response to hydrostatic pressure and that the underlying effect of pregnancy on BBB permeability is due to elevated estrogen levels. Permeability coefficients to Lucifer Yellow (LY) were compared in four groups of Sprague-Dawley rats: NP (n=7), LP (d20; n=7), ovariectomized (OVX; n=6), and estrogen treated OVX (OVX+E; n=7). Posterior cerebral arteries (PCA) were isolated, pressurized in an arteriograph, and perfused with 0.5mg/ml LY in saline. Concentration changes of LY outside the vessel wall were determined at pressures from 60-200 mmHg. The slope of the pressure versus permeability curve is the permeability coefficient to LY. Further, hydraulic conductivity (L_p) was determined in NP (n=6) and LP (n=6) PCAs using a modified Landis technique. The permeability coefficient to LY was increased in LP versus NP animals ($P<0.05$), an effect that may predispose the brain to edema formation during acute hypertension. However, pregnancy did not increase L_p, suggesting that pregnancy differentially affects BBB permeability to LY and water. Estrogen treatment prevented an increase of the permeability coefficient in OVX animals ($P<0.05$), suggesting that estrogen is protective of BBB permeability and that pregnancy increases BBB permeability by a mechanism other than elevated estrogen.

Introduction

Eclampsia is a serious complication of pregnancy accounting for a substantial part of maternal and fetal mortality throughout the world.[1, 2] Eclampsia occurs when the brain is affected by pregnancy-induced hypertension, manifesting with classic neurologic symptoms, including seizures, headache, nausea, vomiting, visual disturbances, and coma.[3]

The primary explanation for the pathogenesis of eclampsia is that it is a form of posterior reversible encephalopathy syndrome (PRES).[4] This syndrome can arise from a sudden increase in blood pressure that causes forced dilatation of the cerebrovasculature, breakthrough of autoregulation, and hyperperfusion. As a result, blood-brain barrier (BBB) disruption can occur, leading to cerebral edema formation.[5, 6] This type of edema, deriving from an unfavorable pressure gradient between vasculature and brain tissue, is called hydrostatic edema and is thought to be the underlying cause of the neurologic symptoms in PRES and eclampsia.[7, 8]

Under normal conditions, the brain is protected against formation of hydrostatic brain edema by the unique features of the BBB. These include the coupling of endothelial cells by high electrical resistance tight junctions, the lack of capillary fenestrations, and a low rate of pinocytosis, resulting in essentially no extravasation of solutes.[9] Together with the very low hydraulic conductivity of the BBB, this arrangement of the BBB makes the effect of hydrostatic pressure on capillary filtration minimal.[10] However, under conditions with acutely elevated blood pressure that causes decreased cerebrovascular resistance, hydrostatic pressure in the cerebrovasculature increases, resulting in BBB permeability. As a consequence, brain edema develops driven by the hydrostatic pressure gradient, i.e. hydrostatic edema.[7, 11]

In a previous study, we found that the blood pressure at which breakthrough of autoregulation occurred was similar in late-pregnant (LP) and nonpregnant (NP) rats. However, only LP rats developed cerebral edema in response to autoregulatory breakthrough,[12] suggesting that pregnancy alone predisposes the brain to edema formation during acute hypertension. Because increases in BBB permeability are considered the major mechanism by which hydrostatic edema forms,[13] this study tested the hypothesis that pregnancy predisposes the brain to edema formation through enhanced cerebral endothelial cell permeability in response to hydrostatic pressure. Therefore, BBB permeability was assessed by determining the permeability coefficient to Lucifer Yellow (LY), as well as the hydraulic conductivity (L_p), in posterior cerebral arteries (PCAs) from NP and LP animals. Permeability coefficients are determined by the regression of the pressure versus permeability curves, allowing for comparison of permeability in response to hydrostatic pressure. The advantage of this in vitro methodology is that it eliminates changes in hemodynamics that can affect the BBB in vivo. Furthermore, since plasma estrogen levels are significantly elevated during pregnancy,[14] it was hypothesized that the underlying effect of pregnancy on the BBB permeability is due to elevated estrogen levels.

To investigate this, the permeability coefficient to LY in PCAs from ovariectomized (OVX) and estrogen treated OVX animals was also assessed.

Materials and methods

Animals

For all experiments female Sprague-Dawley rats (N=45) (Charles River, St. Constant, QU, Canada) were used. All animals were housed in the University of Vermont Animal Care Facility, an American Association for the Accreditation of Laboratory Animal Care (AAALAC) accredited facility. Animals had access to food and water ad libitum and were maintained at a 12 hour light-dark cycle. All the procedures were approved by the University of Vermont Institutional Animal Care and Use Committee and complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Four different groups of rats were used: virgin NP (proestrus stage; n=7 for permeability coefficient experiments and n=6 for L_p experiments), LP (day 20; n=7 for permeability coefficient experiments and n=6 for L_p experiments), OVX with estrogen therapy (OVX+E; n=7), and OVX (n=6). For the OVX+E animals, ovariectomy was performed 14 days before experimentation and pellets (Innovative Research of America, Sarasota, FL, USA) containing 17β-estradiol (0.5 mg 21-day release) and estriol (5.0 mg 21-day release) were subcutaneously implanted in the lateral side of the neck at the time of ovariectomy. For the OVX animals, ovariectomy was performed 28-29 days before experimentation and placebo pellets (Innovative Research of America) were implanted at the time of ovariectomy. All surgical procedures were performed by Charles River (Kingston, NY, USA). An extra group of female rats (n=6) was used to validate the technique for measuring L_p.

Preparation of arteries

The animals were anesthetized with isoflurane in oxygen and decapitated. The brain was quickly removed and placed in physiologic salt solution (HEPES solution). A third-order branch of the posterior cerebral artery (PCA) was carefully dissected, cleared of connective tissue, and placed in an arteriograph chamber (Living Systems, Burlington, VT, USA) for isolated vessel experiments, described below. The PCA was used because cerebral edema in eclampsia is most commonly found in the posterior brain region.[15] For pregnant animals, the abdomen was opened to count the number of pups in utero. For OVX and OVX+E animals the abdomen was opened to verify complete removal of ovaries and to assess the morphologic appearance of the uterus (e.g. edema). For the L_p validation experiments, a PCA and a similar-sized mesenteric artery were dissected from the same animal.

Pressurized arteriograph chamber

A segment of the dissected artery was mounted on two glass cannulas with nylon ties and

pressurized, as previously described.[16, 17] The chamber was attached to a heat exchanger to maintain the temperature of the HEPES solution at 37 ± 0.5ºC and pH at 7.4 ± 0.5. The proximal cannula was attached to an in-line pressure transducer with a peristaltic pump and controller that allowed intravascular pressure to be maintained at a constant pressure or changed at a variable rate. The distal cannula was connected to a peristaltic pump that allowed for flow through the vessel at a set flow rate and intravascular pressure. The whole chamber was placed on an inverted microscope attached to a video camera and monitor. Lumen diameter was measured through an optical window in the bottom of the chamber, using a video dimension analyzer (VDA).

For experiments assessing L_p, some adjustments in the experimental setup described above were made. The peristaltic pump on the distal cannula was removed and the distal cannula closed off so there was no flow through the vessel. Furthermore, the distal end of the vessel was closed off by placing two nylon ties around the vessel before the distal cannula, i.e. the vessel was mounted on the proximal cannula only.

Experimental protocol for permeability coefficient experiments

The arteries were equilibrated for 45 minutes at 60 mmHg after which the HEPES was replaced by fresh HEPES. After perfusing the vessel with 0.5 mg/ml LY-CH (Molecular Probes, Eugene, OR, USA) in HEPES for three minutes, the HEPES outside the vessel wall was sampled to measure baseline fluorescence intensity using a fluorescent spectrophotometer (Photon Technology International, Birmingham, NJ, USA). After 15 minutes at 60 mmHg the HEPES was sampled again to determine the change in fluorescence. The concentration of LY in the HEPES outside the vessel wall was quantified from a linear standard curve plotted from known amounts of LY in HEPES. The HEPES was then replaced by fresh HEPES. This procedure, except for the equilibration step, was repeated at intravascular pressures from 80-200 mmHg in steps of 20 mmHg.

For each animal, intravascular pressure was graphed versus permeability (concentration change of LY during 15 minutes) and a regression line was drawn using Sigmaplot graphing software; the slope of the regression line is the rate of flux.[18] For each group, the average slope, or permeability coefficient to LY, was calculated and an average regression line was drawn.

Measuring hydraulic conductivity using a modified landis technique

To determine L_p, a modified Landis technique was used.[18] This technique was validated by comparing L_p in a mesenteric artery and PCA from the same rat. Briefly, the arteries were equilibrated for 1 hour at 75 mmHg after which the HEPES was replaced by fresh HEPES. After increasing intravascular pressure to 200 mmHg, the vessel was allowed to equilibrate for another 15 minutes. The pressure controller was disconnected from the

servo, allowing the pressure transducer to read intravascular pressure without correcting for the drop in pressure. This allowed determination of fluid filtration by assessing the drop in intravascular pressure over one hour. After this hour, the intravascular pressure was increased back to 200 mmHg, after which the stopcock between the proximal cannula and the transducer was closed and the procedure described above was repeated, allowing measurement of the pressure drop of the transducer alone. This number was subtracted from the intravascular pressure drop found in the first part of the experiment to determine the drop in pressure resulting from fluid filtration in the vessel.

After validation, this technique was used to compare L_p in NP and LP PCAs. One modification was made; the intravascular pressure drop was measured over one hour at 150 mmHg, a pressure that PCAs would experience during breakthrough in vivo.[12, 19]

Determination of estrogen levels

Immediately after the OVX and OVX+E animals were decapitated, trunk blood was collected and serum isolated by centrifuging for 10 minutes at 2500 rpm. Serum was stored at -80 ºC until analysis. Serum 17β-estradiol and estriol levels were determined using commercial ELISA kits (Cayman Chemical, Ann Arbor, MI, USA).

Statistical analysis

Data are presented as mean ± SEM. Differences in body weight, estradiol and estriol serum levels, L_p, and permeability coefficients to LY between NP and LP animals and between OVX and OVX+E animals were determined by one-way ANOVA. Differences were considered significant at $P<0.05$.

Results

The average body weight of the LP animals was significantly greater than that of the NP animals (280.0 ± 6.3 versus 338.6 ± 8.8 g for permeability coefficient experiments and 302.5 ± 8.4 versus 375.0 ± 5.0 g for L_p experiments; $P<0.001$). There was also a significant difference in body weight between OVX and OVX+E animals, with the body weight of OVX animals being greater than that of OVX+E animals (366.7 ± 9.6 versus 238.6 ± 5.9; $P<0.001$). The mean serum 17β-estradiol level of OVX+E animals was 97.1 ± 23.1 pg/mL, which was significantly higher than that of the OVX animals being 23.4 ± 1.2 pg/mL ($P<0.05$). The mean serum estriol level was 778.2 ± 150.9 pg/mL in the OVX+E animals, which was significantly higher compared to 2.9 ± 0.7 pg/mL in the OVX animals ($P<0.001$). It is worth noting that the serum estriol level of the OVX group was below the detection level (8 pg/mL) of the ELISA kit.

Figure 1 shows the permeability coefficients to LY for NP and LP animals. While the basal permeability at 60 mmHg was not significantly different between the groups,

LP animals had a significantly higher permeability coefficient to LY ($x10^3$), which increased 257% in LP compared to NP animals. Therefore, PCAs from LP animals had a significantly greater increase in permeability in response to increased hydrostatic pressure.

Although the permeability coefficient to LY was increased in LP animals, there was no increase in L_p in those animals. Figure 2A shows the L_p in PCAs from NP and LP animals. No significant difference in L_p was found between the groups.

To verify that we could detect a difference in L_p with this technique, we compared a peripheral (mesenteric) vessel to the PCA. Figure 2B shows the L_p in mesenteric arteries and PCAs from female rats. The mesenteric arteries had a L_p of 35.70 ± 5.18 mmHg/hour which was significantly higher than the L_p of the PCAs, being 4.45 ± 1.39 mmHg/hour ($P<0.001$).

To determine the influence of estrogen on permeability, permeability coefficients to LY were compared between OVX and OVX+E animals. Figure 3 shows the permeability coefficients for the OVX and OVX+E group. Again, no significant difference in basal permeability at 60 mmHg between OVX and OVX+E animals was found. However, the OVX animals had a permeability coefficient to LY ($x10^3$) that was significantly increased compared to OVX+E animals ($P<0.05$). In fact, there was a 229% increase in permeability in response to hydrostatic pressure in OVX compared to OVX+E animals.

Discussion

The major findings of this study were that pregnancy significantly increased the permeability coefficient to LY in PCAs, indicating that this state causes increased cerebral endothelial cell permeability in response to hydrostatic pressure. Interestingly, pregnancy did not increase

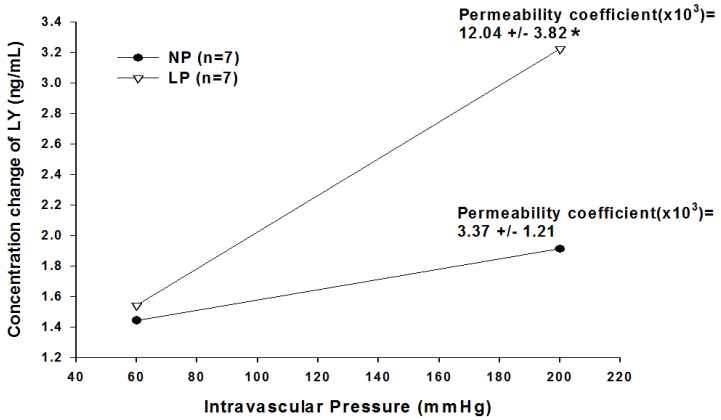

Figure 1. Average regression lines of intravascular pressure versus permeability for posterior cerebral arteries (PCAs) from nonpregnant (NP) and late-pregnant (LP) Sprague-Dawley rats. The slope of these regression lines is the permeability coefficient (x103) to Lucifer Yellow. *P<0.05 versus NP.

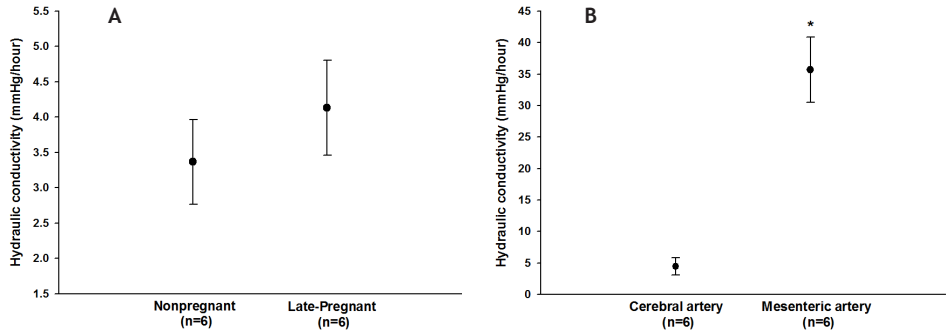

Figure 2. A. Hydraulic conductivity of posterior cerebral arteries (PCAs) from nonpregnant (NP) and late-pregnant (LP) Sprague-Dawley rats at 150 mmHg. **B.** Hydraulic conductivity of posterior cerebral arteries (PCAs) and mesenteric arteries from female Sprague-Dawley rats at 200 mmHg. *P<0.001.

L_p, suggesting a differential effect on permeability to water versus LY. In addition, it does not appear that elevated levels of estrogen during pregnancy are responsible for increased permeability since OVX+E animals had a permeability coefficient to LY more similar to NP than LP animals. In fact, OVX animals had the greatest permeability coefficient to LY of all the groups.

The increased permeability coefficient in PCAs from pregnant animals suggests that pregnancy alone increases BBB permeability in response to hydrostatic pressure. In a previous study, we found that only LP rats developed cerebral edema in response to autoregulatory breakthrough compared to NP animals.[12] Since increases in BBB permeability are considered the major mechanism by which hydrostatic edema forms,[13] the results of the present study suggest that one mechanism behind this predisposition might be increased cerebral endothelial cell permeability.

In contrast to LY, we did not find a difference in L_p between PCAs from NP and LP animals, suggesting that pregnancy does not increase BBB permeability to water. The differential effect of pregnancy on BBB permeability to water and LY suggests that transcellular transport is increased without increasing L_p. In fact, transcellular transport is a primary mechanism of BBB permeability in response to acute hypertension.[20,21] Alternatively, the lack of an increase in L_p during pregnancy may be related to the sensitivity of the L_p measurements. Although we were able to detect a significant difference in L_p between PCAs and mesenteric arteries, this technique may not be sensitive enough to detect a much smaller difference in L_p between NP and LP PCAs.

In order to explore an underlying mechanism by which pregnancy increases the permeability coefficient to LY, we measured permeability coefficients to LY in OVX and OVX+E animals. Since plasma estrogen levels are elevated during pregnancy,[14] we hypothesized that the underlying effect of pregnancy on BBB permeability to LY was due to elevated estrogen plasma levels. Estrogen has been shown to increase BBB permeability

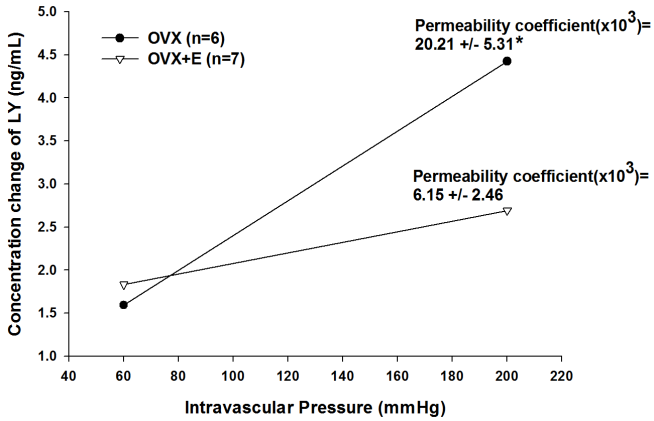

Figure 3. Average regression lines of intravascular pressure versus permeability for posterior cerebral arteries (PCAs) from ovariectomized (OVX) and estrogen treated ovariectomized (OVX+E) Sprague-Dawley rats. The slope of these regression lines is the permeability coefficient (x103) to Lucifer Yellow. *P<0.05 versus OVX+E.

to water,[22] albumin,[23] inulin and sucrose.[24] However, it is not known how estrogen, in plasma levels seen during pregnancy, affects BBB permeability. Interestingly, we found a significantly lower permeability coefficient to LY in OVX+E compared to OVX animals, suggesting that estrogen has a protective effect on BBB permeability. This is in agreement with studies showing that estrogen treatment attenuated edema formation in traumatic brain injury and focal cerebral ischemia.[25, 26] In addition, Bake et al reported decreased Evans Blue extravasation in the olfactory bulb and hippocampus in estrogen treated young adult OVX versus untreated OVX animals.[27] Increased BBB permeability in the cortex, brain stem, and cerebellum was also found by Saija et al. in OVX animals compared to animals in proestrus when estrogen is the lowest.[28] These results suggest that pregnancy increases BBB permeability by a mechanism other than elevated estrogen levels. However, it should be noted that the OVX+E animal model used in this study might not exactly mimic the effects of estrogen during pregnancy because of the large variability in estrogen levels during pregnancy. Animals in this study were treated for 14 days with timed-release pellets which might not have had the same effect of estrogen on BBB permeability as seen during late-pregnancy. Furthermore, pregnancy is a state with numerous physiologic adaptations other than increased estrogen plasma levels. Therefore, the effects of estrogen on BBB permeability may take place in conjunction with other pregnancy related changes that are not seen in OVX+E animals such as changes in sFlt-1 and VEGF levels.[29,30]

Since an unfavorable hydrostatic pressure gradient between the cerebral vasculature and brain tissue is the driving force of hydrostatic edema formation,[7] this study assessed BBB permeability to LY in response to hydrostatic pressure. It is important to note that we did not find a difference in permeability at 60 mmHg, a pressure within the physiologic

range. However, the permeability coefficient for LP animals was significantly increased in response to hydrostatic pressure suggesting that elevated pressure causes greater permeability in pregnancy. LY was chosen as a dye because of its polarity, which means that it does not pass through the high electrical resistance tight junctions of the BBB, and is a marker of transcellular transport.[9] It is therefore likely that pregnancy enhances transcellular transport in response to acute hypertension similar to other studies[20, 21] and that this mechanism underlies the edema formation.

In summary, the present study demonstrated that pregnancy increased the permeability coefficient to LY, an effect that may predispose the brain to hydrostatic edema formation during acute hypertension. However, pregnancy did not increase L_p suggesting that pregnancy differentially affects BBB permeability to LY and water. Furthermore, it was found that estrogen treatment prevented the increase of the permeability coefficient to LY in OVX animals suggesting that estrogen is protective of BBB permeability and that pregnancy increases BBB permeability by a mechanism other than elevated estrogen levels.

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3

REGIONAL EXPRESSION OF AQUAPORIN 1, 4, AND 9 IN THE BRAIN DURING PREGNANCY

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Abstract

Pregnancy is a state of physiologic adaptation, with significant changes in cardiovascular, renal, and hemodynamic systems. Aquaporins (AQPs) may play a role in facilitating these changes. While AQP expression has been assessed in several organs during pregnancy, little is known about its expression in the brain during pregnancy. Therefore, this study assesses the regional expression of AQP1, 4, and 9 during pregnancy and the postpartum period using real-time quantitative polymerase chain reaction. The authors show that AQP1, 4, and 9 are expressed in the anterior and posterior cerebrum, cerebellum, and brainstem of nonpregnant, midpregnant, late pregnant, and postpartum rats. The regional distribution pattern of AQP4 and 9 remained similar during gestation, whereas this pattern changed for AQP1. The expression levels of AQP1, 4, and 9 in the brainstem did not change with gestation, whereas changes were found in the anterior cerebrum for AQP4 and in the posterior cerebrum and cerebellum for all AQPs.

Introduction

Aquaporins (AQPs) are a family of transmembrane channel-forming proteins that facilitate water movement across plasma membranes.[1] In addition to water transport, some AQPs also have permeability to small solutes including glycerol, urea, and monocarboxylates.[2,3] To date, 13 AQPs have been identified in mammalian tissues[4]; however, only 3 have been shown to be expressed in brain in vivo and include AQP1, 4, and 9.[5]

AQP1 is permeable only to water and is expressed in the epithelial cells of the choroid plexus.[6,7] Given this location and the finding that AQP1–deficient mice have reduced cerebrospinal fluid (CSF) formation,[8] AQP1 is proposed to play a role in CSF production.

AQP4 is the most abundant AQP in the brain, and its permeability is restricted to water.[5] Expression of AQP4 is found in several brain regions, including the cortex, hippocampus, magnocellular hypothalamic nuclei, cerebellum, and brainstem.[5,9] In these regions, expression of AQP4 is restricted to the endfeet of astrocytes, bordering the ventricles, the subarachnoid space, and the blood vessels.[10] While some studies have reported AQP4 expression in the endothelium,[11,12] other studies did not find AQP4 at this location.[10,13] Several physiologic roles have been proposed for AQP4. Given its location at the blood-brain and brain-CSF interfaces, it is thought that AQP4 has a role in cerebral water homeostasis. In addition, co-localization in astrocytes with the potassium channel Kir4.1 suggests that AQP4 may be involved in potassium homeostasis.[14] Lastly, AQP4 expression in the magnocellular hypothalamic nuclei suggests a role for AQP4 in central osmoregulation by transferring variations in plasma osmotic pressure from blood to the osmosensitive neurons in these nuclei.[10,15]

AQP9 facilitates the diffusion of both water and several small solutes such as glycerol, urea, purines, pyrimidines, and monocarboxylates.[2] It is expressed in tanycytes, ependymal cells, and astrocytes lining the ventricles, in astrocytes of the glia limitans, and endothelial cells of pial vessels.[16,17] The expression of AQP9 at these locations suggests its involvement in cerebral water homeostasis. Moreover, AQP9 expression is found in catecholaminergic neurons, suggesting a role for AQP9 in brain energy metabolism by facilitating transport of small metabolites such as glycerol and lactate.[17]

We have previously shown that expression of AQP4 protein is increased in the brain from late pregnant rats.[18] Pregnancy is a state of physiologic adaptation during which significant changes in cardiovascular, renal, and hemodynamic systems occur.[19,20] These changes include a 45% increase in plasma volume and a 30% to 50% increase in cardiac output.[19] How AQP expression changes during pregnancy to facilitate these adaptations has been studied in several organs. For example, AQP2 mRNA and protein expression are shown to increase more than 100% in rat kidneys during pregnancy.[21] This upregulation of AQP2 may contribute to the water retention seen in pregnancy. Furthermore, the expression profile of AQP0 through 9 has been assessed in mice uteri during the peri-implantation period since these AQPs may participate in the preparation of the uterus for implantation

by facilitating the formation of uterine edema.[22] While the effect of pregnancy on these organ systems has been well studied, how pregnancy affects the brain, including AQP expression, is not well understood. Our previous study investigated AQP4 expression in the brain during pregnancy and found a significant increase in AQP4 protein in the whole brain. However, nothing is known about the regional distribution during pregnancy or how the expression of other AQPs in the brain changes during gestation. Therefore, this study investigates the expression of AQPs 1, 4, and 9 in different brain regions during pregnancy and the postpartum state using real-time quantitative polymerase chain reaction (RQ-PCR).

Materials and methods

Animals

For all experiments, female Sprague-Dawley rats (Charles River, St Constant,QC, Canada) were used. All animals were housed in the University of Vermont Animal Care Facility, a facility accredited by the American Association for the Accreditation of Laboratory Animal Care. Animals had access to food and water ad libitum and were maintained at a 12-hour light/dark cycle. All of the procedures were approved by the University of Vermont Institutional Animal Care and Use Committee and complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Four different groups of animals were studied: virgin nonpregnant (NP; proestrus stage), midpregnant (MP; day 10 of a 22-day gestation), late pregnant (LP; day 20), and postpartum (PP; day 4).

Real-time quantitative reverse transcription PCR

To obtain tissue for real-time quantitative reverse transcription PCR (RQ-PCR) analysis, a sample size of 4 to 6 rats was used for each group. Animals were anesthetized with isoflurane (Abbott, North Chicago, IL) and decapitated. Brains were quickly removed from the skull and split into right and left hemispheres. The cerebral cortex, cerebellum, and brainstem were separated from each other; the cerebral cortex was further divided into the anterior and posterior cerebrum by a coronal cut at the level of the optic chiasm. Sections were snap frozen in liquid nitrogen and stored at –80°C until analysis.

For total RNA extraction, brain sections were placed in a FastPrep instrument (MP Biomedicals, Solon, OH) for 45 seconds after adding 1 ml TRIzol Reagent (Invitrogen, Carlsbad, CA) and 0.2 g silicon carbide and aluminum oxide mix (Washington Mills, Niagara Falls, NY). Following centrifugation at 12000 rcf for 10 minutes at room temperature, cleared homogenate solutions were incubated for 5 minutes at room temperature. After addition of 0.2 mL of chloroform, samples were shaken for 15 seconds, incubated for 2 to 3 minutes at room temperature, and centrifuged for 15 minutes under the same conditions. RNA was precipitated from the aqueous phase by adding 0.5 mL isopropyl alcohol, incubating for 10 minutes at room temperature, and then centrifuging for 30 minutes at 4°C. After washing with 1 mL 75% ethanol, RNA pellets were briefly dried and finally dissolved in RNAse-

free water. Isolated RNA was then quantified using the NanoDrop spectrophotometer and checked for integrity in a Bioanalyzer (Bio-Rad, Hercules, CA).

Synthesis of cDNA was accomplished by adding 2 µg of total RNA, 1 µL of 50 µM random primers (ABI Research, Oyster Bay, NY), and 1 µL 10 µM dNTP Mix (Invitrogen) in a 13 µL total volume. The mixture was heated for 5 minutes at 65°C and incubated on ice for at least 1 minute. Next, 4 µL 5X First-Strand Buffer (Invitrogen), 1 µL 0.1 M dithiothreitol (Invitrogen), 1 µL RNaseOUT Recombinant RNase Inhibitor (Invitrogen), and 1 µL of Superscript III RT (Invitrogen) were added to the reaction. Following incubation for 5 minutes at 25°C, the temperature was increased to 50°C for another 30 minutes. The reaction was inactivated by heating to 70°C for 15 minutes.

The resulting reverse-transcribed cDNA was amplified using Assays-on-Demand (AOD) gene expression products kits for AQP1, 4, and 9 (assay IDs Rn00562834_m1, Rn00563196_m1, and Rn00576331_m1; Applied Biosystems, Foster City, CA). One microliter of cDNA was mixed with 10 µL TaqMan Universal PCR Master Mix (Applied Biosystems), 1 µL AOD, and 8 µL water. The PCR was carried out in a 7900HT Sequence Detection System (Applied Biosystems) for 2 minutes at 50°C and 10 minutes at 95°C followed by 15 seconds at 95°C and 1 minute at 40°C for 40 cycles. MapK6 was used as an endogenous control. All samples were run in technical duplicates. Data were analyzed using Sequence Detection 2.2 software (Applied Biosystems).

Comparison of groups

Two sets of normalization were done to assess both the regional distribution of AQPs at each gestational stage and also how gestation affected expression in the different brain regions. To assess the regional distribution of AQP expression at different gestational ages, data were normalized to the values found for the anterior cerebrum for each AQP in each gestational group. To assess how gestation affected the expression of AQPs within the brain regions, data were normalized to the NP values for each AQP in each region.

Statistical analysis

All data are expressed as the mean ± SEM. Differences in AQP mRNA expression were determined using a Wilcoxon signed-rank test with comparison to a hypothetical value. Differences between unnormalized data were determined using an analysis of variance with a post hoc Student-Newman-Keuls test for multiple comparisons. Differences were considered significant if $P<.05$.

Results

Regional distribution of AQPs

To assess the regional distribution of AQPs, expression levels were normalized to the levels found for the anterior cerebrum (Figures 1, 2, and 3).

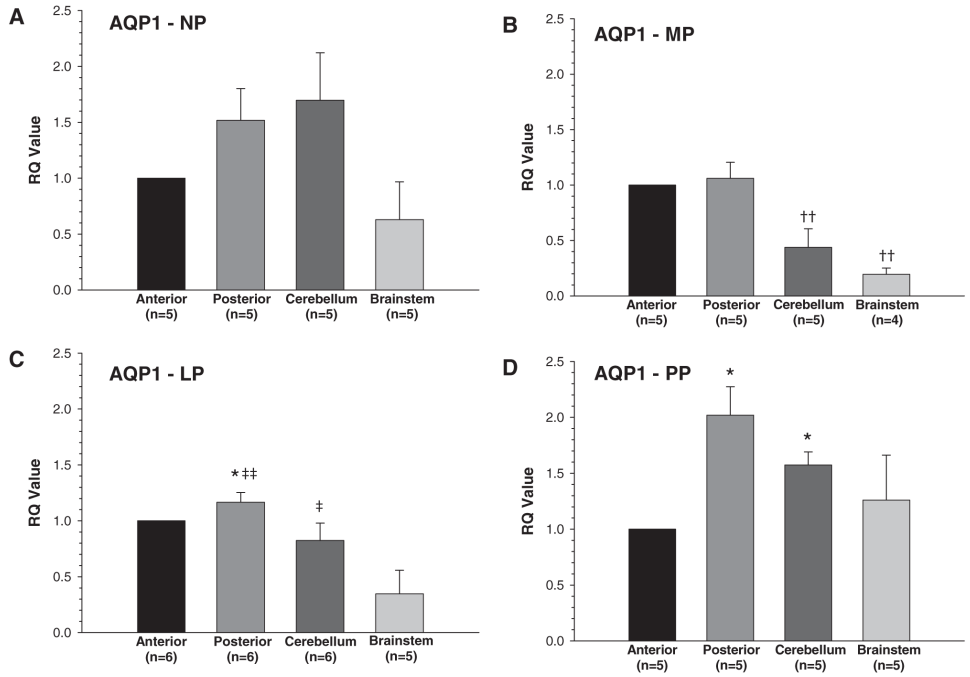

Figure 1. Graphs showing regional aquaporin 1 (AQP1) mRNA distribution between anterior and posterior cerebrum, cerebellum, and brainstem in brains from **(A)** nonpregnant (NP), **(B)** midpregnant (MP; day 10), **(C)** late-pregnant (LP, day 20), and **(D)** postpartum (PP; day 4) rats. AQP1 expression was normalized to the expression found in the anterior cerebrum.
**P*<.05 versus anterior. ††*P*<.01 vs posterior. ‡*P*<.05 vs brainstem. ‡‡*P*<.01 versus brainstem.

Regional distribution of AQP1

AQP1 was expressed in all brain regions in all 4 groups of animals (Figure 1). The NP and PP animals showed a similar distribution pattern in the brain, with higher expression in the posterior cerebrum and cerebellum compared with the anterior cerebrum (Figure 1A and D). However, this was significant only in the PP group (Figure 1D; *P*<.05 vs anterior). The MP animals had lower expression in the cerebellum and brainstem compared with the posterior cerebrum (Figure 1B; *P*<.01). The LP animals also had lower expression in the brainstem compared with the posterior cerebrum (Figure 1C; *P*<.01). In addition, the posterior cerebrum revealed higher expression versus the anterior cerebrum (*P*<.05), and there was more expression in the cerebellum compared with the brainstem (*P*<.05).

Regional distribution of AQP4

AQP4 was also expressed in all brain regions in all 4 groups of animals (Figure 2). Unlike the distribution of AQP1, the distribution pattern of AQP4 within the brain was similar in all groups. All groups showed the highest expression in the brainstem (Figure 2A, NP: *P*<.05 vs anterior and posterior; Figure 2B, MP: *P*<.01 vs anterior and posterior, *P*<.05 vs cerebellum;

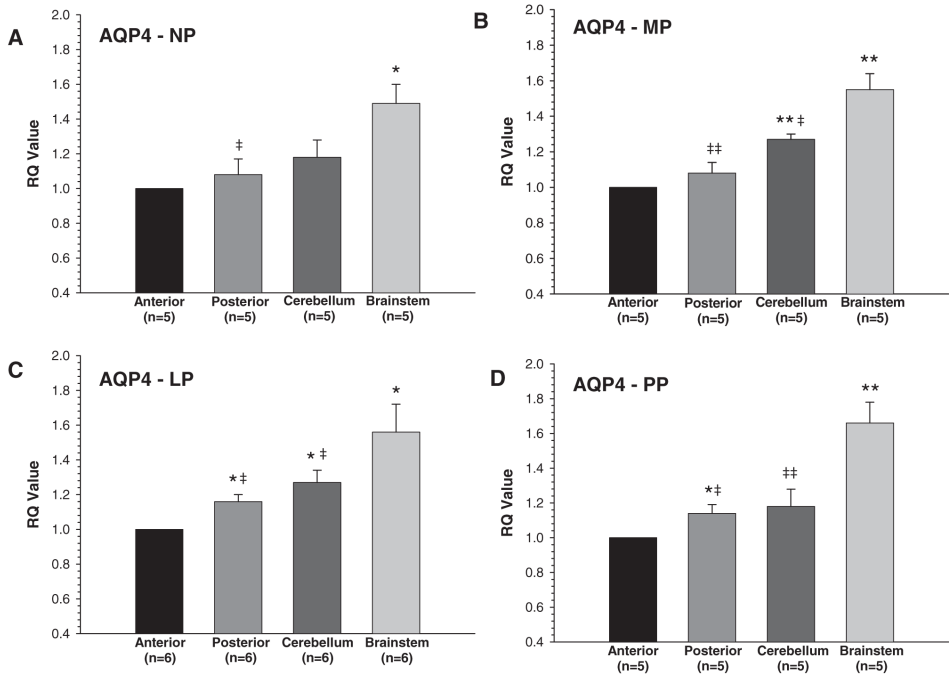

Figure 2. Graphs showing regional aquaporin 4 (AQP4) mRNA distribution between anterior and posterior cerebrum, cerebellum, and brainstem in brains from **(A)** nonpregnant (NP), **(B)** midpregnant (MP; day 10), **(C)** late pregnant (LP; day 20), and **(D)** postpartum (PP; day 4) rats. Expression of AQP4 was normalized to the expression found in the anterior cerebrum.
**P*<.05 vs anterior. ***P*<.01 versus anterior. ‡*P*<.05 versus brainstem. ‡‡*P*<.01 versus brainstem.

Figure 2C, LP: *P*<.05 vs anterior, posterior, and cerebellum; Figure 2D, PP: *P*<.01 vs anterior and cerebellum, *P*<.05 vs posterior), followed by the cerebellum (Figure 2B, MP: *P*<.01 vs anterior; Figure 2C, LP: *P*<.05 vs anterior), posterior cerebrum (Figure 2C and D, LP and PP: *P*<.05 vs anterior), and anterior cerebrum.

Regional distribution of AQP9

AQP9 was also expressed in all brain regions in all 4 groups of animals (Figure 3). Similar to AQP4, the regional distribution of AQP9 in the brain was similar in all groups, with no significant difference between the anterior and posterior cerebrum and less expression in both the cerebellum (Figure 3A, NP: *P*<.05; Figure 3B, MP:*P*<.05 vs anterior, *P*<.01 vs posterior; Figure 3C, LP: *P*<.05; Figure 3D, PP: *P*<.05 vs anterior, *P*<.01 vs posterior) and brainstem compared with the anterior and posterior cerebrum (Figure 3A, NP: *P*<.05; Figure 3B, MP: *P*<.05 vs anterior, *P*<.01 vs posterior; Figure 3D, PP: *P*<.05 vs posterior).

Gestational changes in AQP expression

To assess how gestation affected the expression of AQPs within the brain regions, data

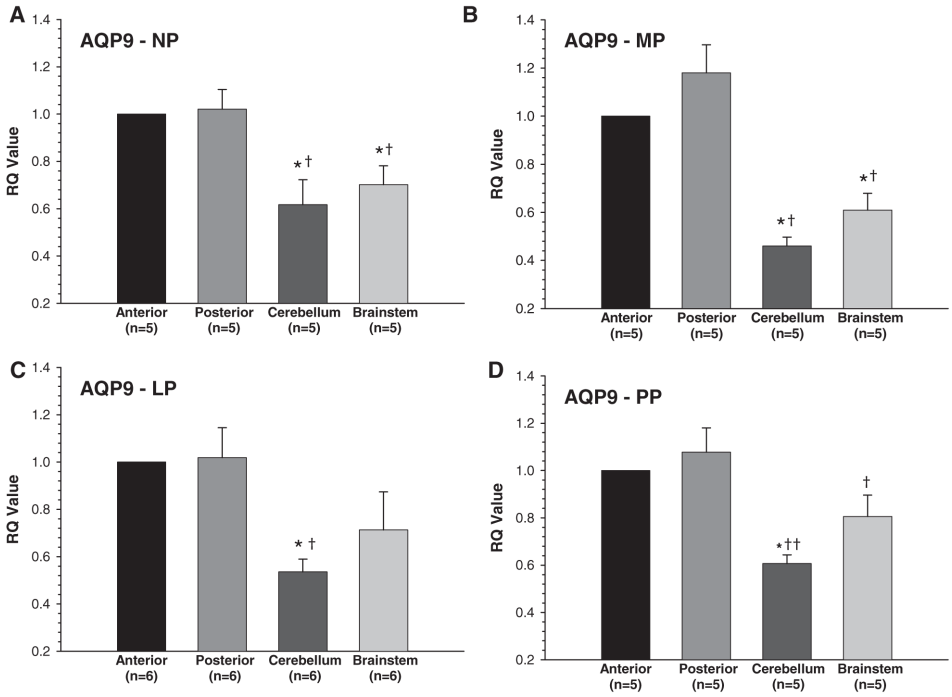

Figure 3. Graphs showing regional aquaporin 9 (AQP9) mRNA distribution between the anterior and posterior cerebrum, cerebellum, and brainstem in brains from **(A)** nonpregnant (NP), **(B)** midpregnant (MP; day 10), **(C)** late pregnant (LP; day 20), and **(D)** postpartum (PP; day 4) rats. AQP9 expression was normalized to the expression found in the anterior cerebrum.
**P*<.05 versus anterior. †*P*<.05 versus posterior. ††*P*<.01 versus posterior.

were normalized to the NP values for each AQP in each region (Figures 4, 5, and 6).

Gestational changes in AQP1 expression

AQP1 expression compared to NP animals did not significantly change with gestation in the anterior cerebrum and brainstem (Figure 4A and D). However, expression did change significantly in the posterior cerebrum and cerebellum (Figure 4B and C). In the posterior cerebrum, expression decreased in MP and LP compared with NP animals (*P*<.05). Expression in the cerebellum was lower in MP, LP, and PP compared with NP animals (*P*<.05), with the expression in the PP animals being higher than in the MP animals (*P*<.01).

Gestational changes in AQP4 expression

AQP4 expression compared to NP animals in the anterior cerebrum did not change in pregnant animals but was lower in PP animals compared with NP animals (Figure 5A; *P*<.05). Expression increased in both the posterior cerebrum and cerebellum in MP and LP animals compared to NP animals (Figure 5B and C; *P*<.05). In the brainstem, no changes in

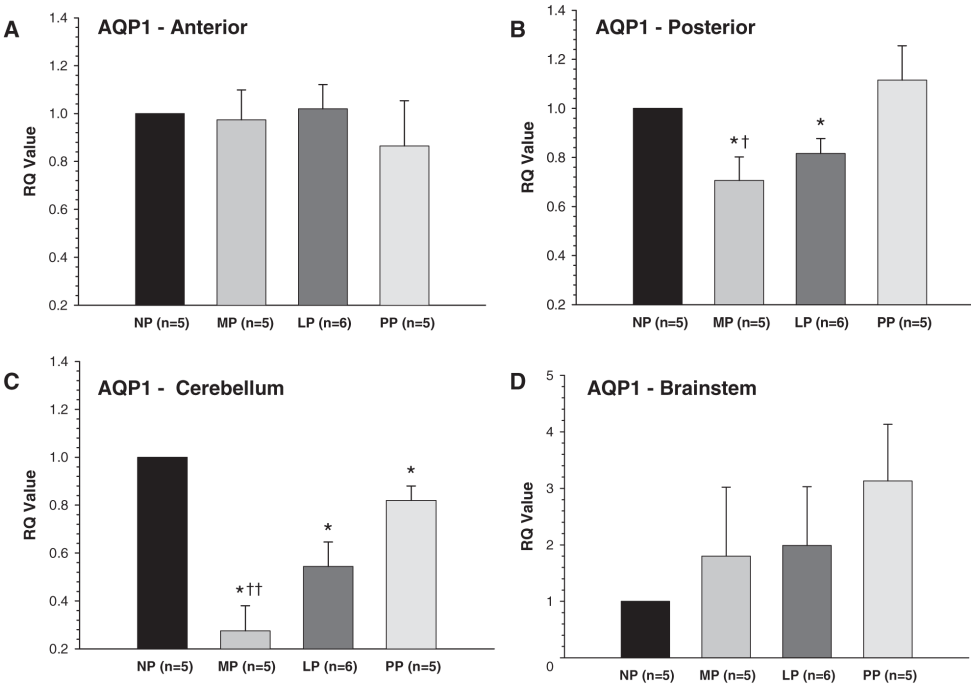

Figure 4. Graphs showing aquaporin 1 (AQP1) mRNA expression levels at different gestational ages in **(A)** anterior **(B)** posterior cerebrum, **(C)** cerebellum, and **(D)** brainstem. Expression of AQP1 was normalized to the expression found in nonpregnant (NP) animals.
*P<.05 versus NP. †P<.05 versus postpartum (PP). ††P<.01 vs PP. LP indicates late pregnant; MP, midpregnant.

expression were seen with gestation (Figure 5D).

Gestational changes in AQP9 expression

No changes in AQP9 expression with gestation were found in the anterior cerebrum and brainstem (Figure 6A and D). However, expression did change with gestation in the posterior cerebrum and cerebellum (Figure 6B and C). In the posterior cerebrum, expression was decreased in LP versus NP animals (P<.05). In the cerebellum, expression decreased in both MP and LP compared with NP animals (P<.05) and increased in PP versus MP and LP animals (P<.01 vs MP; P<.05 vs LP).

Discussion

In this study, we assessed the expression of AQPs 1, 4, and 9 in different brain regions during pregnancy and the postpartum state using real-time quantitative PCR. We showed that AQP 1, 4, and 9 are expressed in the anterior and posterior cerebrum, cerebellum, and brainstem of NP, MP, LP, and PP rats. The regional distribution pattern of AQP4 and 9

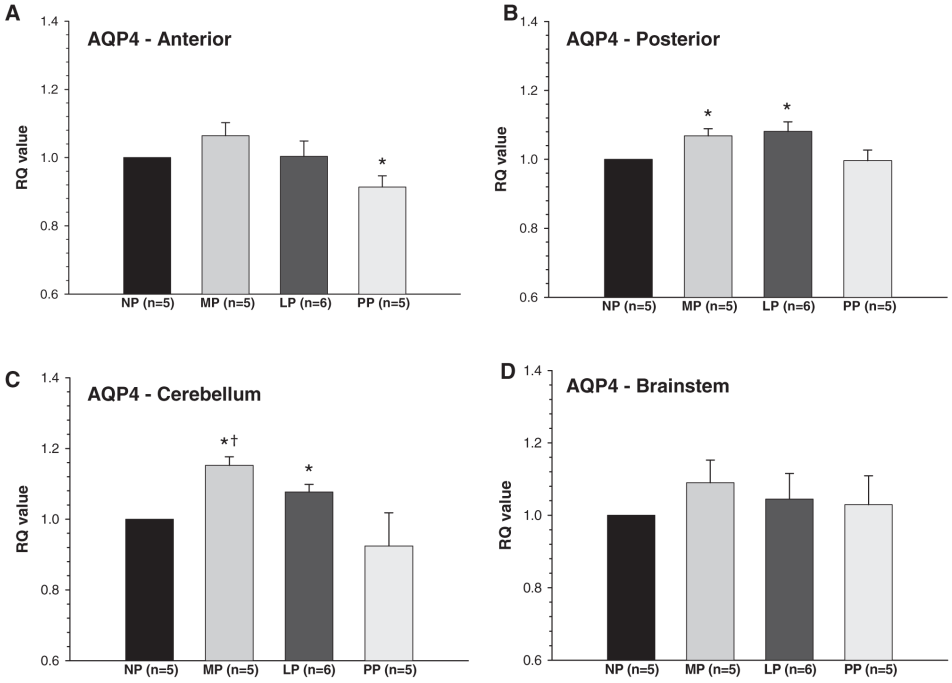

Figure 5. Graphs showing aquaporin 4 (AQP4) mRNA expression levels at different gestational ages in **(A)** anterior and **(B)** posterior cerebrum, **(C)** cerebellum, and **(D)** brainstem. Expression of AQP4 was normalized to the expression found in nonpregnant (NP) animals.
*P<.05 versus NP. †P<.05 versus postpartum (PP). LP indicates late pregnant; MP, midpregnant.

remained similar during gestation, whereas this pattern changed for AQP1. The expression levels of AQP1, 4, and 9 in the brainstem did not change with gestation, whereas changes were seen in the anterior cerebrum for AQP4 and in the posterior cerebrum and cerebellum for all AQPs. To our knowledge, this is the first study to assess the regional distribution of AQPs 1, 4, and 9 in the brain during pregnancy.

AQP1 mRNA expression was found in all brain regions, namely, the anterior and posterior cerebrum, cerebellum, and brainstem in all groups (Figure 1), and AQP1 is thought to play a role in the formation of CSF. This is suggested by its location in the epithelial cells of the choroid plexus[6,7] and the finding that AQP1-deficient mice have reduced CSF formation.[8] Since the choroid plexus is located in the posterior cerebrum, finding AQP1 in all brain regions suggests that AQP1 expression is not restricted to the choroid plexus epithelium. This is in agreement with results from Dolman et al.,[23] who showed the presence of AQP1 mRNA and protein at very low levels in primary rat brain microvessel endothelial cells in culture.

Even though we found AQP1 expression in all brain regions, the relative level of expression is not known. In the NP animals, no difference in expression was found between the regions, although the distribution showed a similar pattern to that of the PP animals,

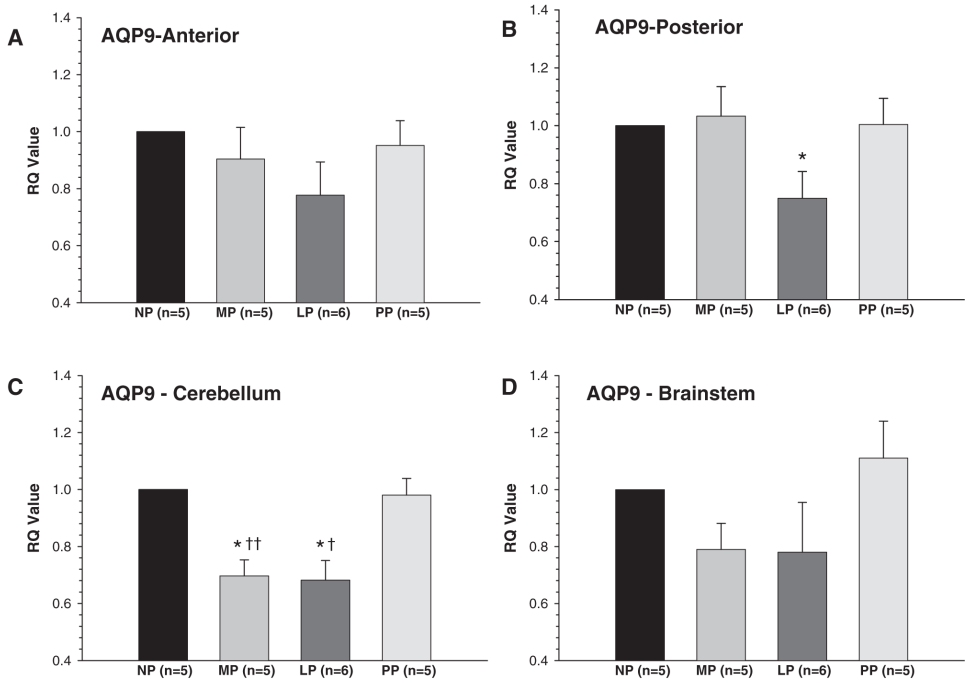

Figure 6. Graphs showing aquaporin 9 (AQP9) mRNA expression levels at different gestational ages in **(A)** anterior and **(B)** posterior cerebrum, **(C)** cerebellum, and **(D)** brainstem. Expression of AQP9 was normalized to the expression found in nonpregnant (NP) animals. **P*<.05 versus NP. †*P*<.05 versus postpartum (PP). ††*P*<.01 versus PP. LP indicates late pregnant; MP, midpregnant.

with higher expression in both the posterior cerebrum and cerebellum compared with anterior cerebrum. The MP animals showed a different pattern, with no difference between the anterior and posterior cerebrum and decreased expression in both the cerebellum and brainstem compared with the posterior cerebrum. In LP animals, a distribution pattern with higher expression in the posterior versus anterior cerebrum and the lowest expression level in the brainstem compared with posterior cerebrum and cerebellum was found. Together, these results suggest that pregnancy changed the distribution pattern of AQP1 regionally in the brain. The significance of this is not clear for this study, and further studies are needed to assess any functional consequence of this gestation-induced redistribution of AQP1.

When assessing gestational changes in AQP1 expression (Figure 4), no significant differences were seen in the anterior cerebrum and brainstem. However, although not significant, the mean expression level in the brainstem increased ~2- to 3-fold during pregnancy and the postpartum period, suggesting that pregnancy does affect the expression of AQP1 in this region. In both the posterior cerebrum and cerebellum, a significant decrease in AQP1 expression was found in brains from MP and LP versus NP animals. Since AQP1 is thought to be involved in CSF formation, the decrease in expression noted in the posterior region during pregnancy may have an influence on this CSF formation. However,

it is also possible that expression changes take place at locations other than the choroid plexus since AQP1 expression was found in all brain regions.

AQP4 is the most abundant AQP in the brain and is expressed in several brain regions, including the cortex, hippocampus, magnocellular hypothalamic nuclei, cerebellum, and brainstem.[5,9] This is in agreement with the current study, which shows AQP4 expression in the anterior and posterior cerebrum, cerebellum, and brainstem (Figure 2). The same distribution pattern was seen between these brain regions in all gestational groups. AQP4 expression was lowest in the anterior cerebrum, followed by the posterior cerebrum, cerebellum, and brainstem. This expression in the anterior cerebrum and brainstem did not change significantly with gestation, except for a decrease in the anterior cerebrum in PP animals (Figure 5). In the posterior cerebrum and cerebellum, however, expression did change during pregnancy. In both MP and LP animals, increased expression of AQP4 was found in these regions. This is in agreement with our previous study, which found that AQP4 protein expression was increased in LP animals in the whole brain.[18]

AQP4 is thought to have several functions in the brain. Given its location at the blood-brain and brain-CSF interfaces, it is thought that AQP4 has a role in brain water homeostasis and in cerebral edema formation and resolution under pathologic conditions.[24-27] In several brain disorders associated with cerebral edema, regulation of the level of AQP4 expression was found, including cerebral ischemia,[24-27] brain tumors,[28] and brain trauma.[29-31] Furthermore, a study using AQP4 knockout mice found that these mice had less edema following acute water intoxication and ischemic stroke compared with wild type mice, suggesting involvement of AQP4 in edema formation.[32] On the other hand, involvement of AQP4 in the resolution of vasogenic edema has also been suggested.[33] Thus, although the role of AQP4 in cerebral edema formation is not completely clear, being the most abundant AQP in the brain, its role in edema formation may be important under pathologic conditions.

One pathologic condition during pregnancy that involves cerebral edema formation is posterior reversible encephalopathy syndrome and eclampsia. Eclampsia is a serious complication of pregnancy in which neurologic symptoms arise from the development of vasogenic brain edema following an acute elevation of blood pressure.[34] Since edema formation in eclampsia is mainly found in the posterior brain regions,[35,36] it is interesting that we found higher expression of AQP4 in the posterior versus the anterior cerebrum in both LP and PP animals, 2 states during which eclampsia usually develops.[34,37]

AQP4 is also expressed in the magnocellular nuclei of the hypothalamus, and a role for this water channel has been proposed in central osmoregulation by transferring variations in plasma osmotic pressure from blood to the osmosensitive neurons in these nuclei.[10,15] However, we did not find any changes in expression in the brainstem, where the hypothalamus is located, with gestation.

AQP9 is the only aquaporin in the brain that facilitates diffusion of both water and

several small solutes such as glycerol, urea, purines, pyrimidines, and monocarboxylates.[2] Expression is found in tanycytes, ependymal cells and astrocytes lining the ventricles, astrocytes of the glia limitans, and endothelial cells of pial vessels.[16,17] Like AQP4, this localization suggests involvement of AQP9 in cerebral water homeostasis. Moreover, AQP9 expression is found in catecholaminergic neurons, suggesting a role for AQP9 in brain energy metabolism by facilitating transport of small metabolites such as glycerol and lactate.[17]

We found AQP9 in all brain regions investigated, corresponding to the locations described previously (Figure 3). All gestational groups revealed a similar pattern of distribution between the regions, showing no significant difference between the anterior and posterior cerebrum and a lower expression level in both the cerebellum and brainstem compared with the anterior and posterior cerebrum. When assessing the changes in AQP9 expression with gestation (Figure 6), no significant changes were found in the anterior cerebrum and brainstem. However, expression in the posterior cerebrum decreased in LP versus NP animals, and in the cerebellum, expression was decreased in MP and LP versus NP. These decreased levels went back to the NP levels in PP animals, suggesting a direct effect of pregnancy on this AQP.

It is worth noting that we assessed the expression of AQPs 1, 4, and 9 using RQ-PCR, which is the most sensitive and accurate method to assess changes in mRNA expression. While other techniques such as Western analysis for protein levels and immunohistochemistry may provide further information regarding changes in AQPs in the brain during gestation, the results from this study provide a basis of our understanding of how pregnancy alters AQP gene expression in the brain. Because of the large number of groups and brain regions, we did not pursue other techniques but focused on expression level changes for which other studies can follow.

Acknowledgement

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4

STRUCTURE AND FUNCTION OF CEREBRAL AND MESENTERIC RESISTANCE ARTERIES IN LOW-DOSE ENDOTOXIN-INFUSED PREGNANT RATS

Submitted

Abstract

Objective

Since the cerebrovasculature likely plays a prominent role in the pathophysiology of eclampsia, we assessed effects of low-dose endotoxin-induced experimental preeclampsia on function and structure of rat posterior cerebral arteries (PCA) and mesenteric arteries (MA).

Methods

Nonpregnant (NP) and pregnant (P) rats were infused with saline (NP-CTL, n=9; P-CTL, n=9) or low-dose endotoxin (NP-endotoxin, n=9; P-endotoxin, n=10). Myogenic activity, pressure of forced dilatation (FD) and structural properties were evaluated in PCA and MA.

Results

PCA underwent FD between 125-150 mmHg in P-endotoxin (repeated measures ANOVA versus 75 mmHg; $P<0.05$) and between 150-175 mmHg in P-CTL and NP animals (repeated measures ANOVA versus 75 mmHg; $P<0.05$). PCA myogenic tone was unaffected by pregnancy or endotoxin, however, pregnancy decreased MA myogenic tone ($P<0.05$ versus NP). Passive characteristics of PCA and MA were unaffected by pregnancy or endotoxin.

Conclusion

Low-dose endotoxin-infusion during pregnancy, but not pregnancy alone, decreased pressure of FD in PCA. This may predispose to cerebral autoregulatory breakthrough and edema formation during increased blood pressure as seen in eclampsia.

Introduction

Preeclampsia can affect several maternal organs, including the brain in the form of eclampsia.[1] The pathophysiology of eclampsia is still largely unclear but is considered to be a form of the posterior reversible encephalopathy syndrome (PRES).[2] This syndrome can arise from a sudden increase in blood pressure that causes forced dilatation of the cerebrovasculature and breakthrough of autoregulation.[3,4] As a result, blood-brain barrier disruption can occur, leading to cerebral edema formation which is thought to be the underlying cause of the neurological symptoms of PRES/eclampsia.[3,4] Interestingly, eclamptic patients may show minimal hypertension, suggesting pregnancy or preeclampsia induced changes in the cerebrovasculature, which predispose to cerebral edema development.[5,6]

Several studies have focussed on the effect of pregnancy and preeclampsia on the peripheral vasculature. For example, pregnancy has been shown to decrease myogenic reactivity and blunt responses to vasoconstrictor agents in rats.[7-10] While these changes may add to the decreased peripheral resistance during pregnancy, preeclampsia seems to inhibit these adaptations to pregnancy. For instance, mesenteric arteries (MA) from the reduced uterine perfusion pressure model of preeclampsia have been shown to have increased myogenic reactivity and responses to vasoconstrictor agents compared to healthy pregnant rats.[11,12]

Despite a large body of knowledge on the effect of pregnancy and preeclampsia on the peripheral vasculature, only few studies have focussed on pregnancy-induced adaptations of the cerebral vasculature. In rat posterior cerebral arteries (PCA), Cipolla et al have demonstrated that pregnancy diminishes myogenic tone and decreases the pressure at which forced dilation (FD) occurs.[13,14] Furthermore, hypertension and low-dose endotoxin treatment have been shown to have differential effects in pregnant and nonpregnant animals.[14-16] For example, during pregnancy, in contrast to the nonpregnant situation, hypertension does not induce protective remodeling of PCA.[14,15] These findings suggest that during pregnancy, the cerebrovasculature may be more vulnerable to breakthrough of autoregulation and edema formation.

It is of particular interest to explore how preeclampsia affects the cerebral blood vessels and predisposes to PRES. Therefore, this study assesses the function and structure of PCA in a well established preeclampsia rat model, the low-dose endotoxin-infused pregnant rat.[17-20] In this model, a low grade inflammatory response results in hypertension, proteinuria and disseminated intravascular coagulation similar to human preeclampsia.[17,21] As a control, we used pregnant rats infused with saline. To evaluate the effect of pregnancy, we also included nonpregnant rats infused with either endotoxin or saline. Furthermore, to investigate whether low-dose endotoxin infusion and pregnancy influence the cerebral and systemic circulation similarly, simultaneous experiments were performed in MA.

Materials and methods

Animals

Female Wistar outbred rats (Harlan Inc, Horst, the Netherlands), 200-220 grams, were used for all experiments. Animals had access to food and water ad libitum and were maintained at a 12 hour light-dark cycle. All animal experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Committee for Animal Experiments of the University of Groningen.

Vaginal smears were taken daily to follow estrous cyclicity until selection for experiments. Rats were rendered pregnant by housing them on pro-estrus with fertile male Wistar rats for 1 night. When spermatozoa were detected in the vaginal smear the next day, this was designated as day 0 of pregnancy.

In cyclic and (day 0) pregnant rats a cannula was surgically inserted into the right jugular vein under isoflurane/oxygen anaesthesia according to standard methods.[22] This cannula allows stress-free infusion of saline or endotoxin.

As described previously,[18] endotoxin (*Escherichia coli*, 0.55:B5, Whittaker MA Bioproducts inc., Walkerville, MD) (1.0 µg/kg body weight) was dissolved in 2 ml pyrogen-free saline and infused through the jugular vein cannula, using an infusion pump at an infusion rate of 2.0 ml/hour.

Pregnant rats were infused on day 14 of pregnancy with either saline (P-CTL) or endotoxin (P-endotoxin). Nonpregnant rats received infusion of either saline (NP-CTL) or endotoxin (NP-endotoxin) 14 days after cannulation. The low-dose endotoxin-treated pregnant rat is an established model of preeclampsia, characterized by hypertension, proteinuria, disseminated intravascular coagulation, generalized activation of the inflammatory response and endothelial cell activation.[17-20] Recently, also aortic endothelial dysfunction has been demonstrated in these rats.[23] The development of the preeclamptic-like syndrome in this model is considered to result from a systemic inflammatory response induced by endotoxin.[17,21,24] In addition to studies focussing on the pathophysiology of preeclampsia, studies concerning therapeutic options for preeclampsia have also been performed in this model.[20,25,26]

Solutions

Krebs solution of the following composition was used for the vessel experiments (mM): 120 NaCl, 5.9 KCl, 25.2 $NaHCO_3$, 1.2 NaH_2PO_4, 10.4 glucose, 1.21 $MgCl_2 \bullet 6H_2O$ and 2.52 $CaCl_2$ (E. Merck Darmstadt, Germany).

Pressurized arteriograph system

Six days after infusion, pregnant (day 20 of pregnancy) and nonpregnant rats were anesthetized with isoflurane in oxygen and decapitated. The brain and part of the mesentery were quickly removed and placed in ice cold Krebs. Third-order branches of

the PCA and MA were carefully dissected, cleared of connective tissue, and transferred to an arteriograph chamber for pressurized arteries (Living Systems, Burlington, VT, USA). Then they were mounted on two glass micropipettes with nylon ties and pressurized, as previously described.[13] The arteriograph chamber was continuously recirculated with warmed (37.0±0.5°C) and oxygenated (5% CO_2 in O_2) Krebs solution with a pH of 7.4.

The proximal cannula was attached to an in-line pressure transducer with a peristaltic pump and controller that adjusted intravascular pressure. The distal cannula was closed off to eliminate flow-induced responses (blind sac). To determine lumen diameter and wall thickness, a video dimension analyzer (Living Systems, Burlington, VT, USA), attached to a monitor, was used. On the monitor, the artery in the arteriograph chamber was shown, using a video camera connected to an inverted microscope.

Experimental protocol

For active measurements, arteries were allowed to equilibrate for 45 minutes at 50 mmHg for PCA and 60 mmHg for MA, after which Krebs was refreshed. For PCA, intravascular pressure was then increased in 25 mmHg increments to 175 mmHg. For MA, intravascular pressure was set at 40 mmHg and increased in 20 mmHg increments to 160 mmHg. After maintaining each pressure step for ~10 minutes to reach a stable contractile response, lumen diameter and wall thickness were measured.

Following myogenic protocols, calcium-containing Krebs was replaced with calcium-free Krebs supplemented with ethyleneglycol-bis-(b-aminoethylether)tetraacetic acid (EGTA, 2 mmol/l) to obtain passive measurements of lumen diameter and wall thickness of PCA and MA at pressures from 5-175 mmHg and 5-160 mmHg, respectively.

Data calculations

The active measurement myogenic tone was calculated as percent decrease in active diameter from the maximally dilated diameter in calcium-free Krebs at each intravascular pressure, i.e., myogenic tone (%) = $[(D_{Ca\text{-}free} - D_{Ca})/D_{Ca\text{-}free}] \times 100\%$, where D is the diameter of the vessel in calcium-containing (D_{Ca}) and calcium-free ($D_{Ca\text{-}free}$) Krebs.

The passive measurement percent distensibility was calculated at each pressure in completely relaxed vessels in calcium-free Krebs by determining diameter changes as a function of pressure, i.e., distensibility (%) = $[(D_{pressure}/D_{5mmHg)} - 1] \times 100\%$, where $D_{pressure}$ is the diameter of the vessel at a certain pressure and D_{5mmHg} is the diameter of the vessel at 5 mmHg.

Passive circumferential wall stress was calculated by the equation T/ω, where ω is wall thickness and T is circumferential wall tension. T is calculated as $T=p \times r$, where p is pressure in dynes per square centimeter (1 mmHg = 1333.2 dynes/cm^2) and r is the radius in centimeter.

Statistical analysis

Statistical analysis was performed using SPSS for Windows 18.

Differences in number and length of pups between P-CTL and P-endotoxin were assessed using Student's t-tests. To determine the effect of pregnancy or endotoxin infusion, differences in body weight, wall thickness and wall-to-lumen ratio were determined by two-way ANOVA followed by a post hoc Bonferroni test for multiple comparisons where appropriate. Furthermore, linear regression analysis was performed to assess the slope of the myogenic tone curve of each experimental group and to determine whether this slope significantly differed from zero. This was executed in the intravascular pressure range of 50-150 mmHg for PCA and 40-140 mmHg for MA.

To evaluate changes in active diameter for different pressures, repeated measures ANOVAs were performed for each experimental group. We tested whether active diameter changed with pressures below or above 75 mmHg for PCA or below or above 80 mmHg for MA, pressures that these vessels are exposed to under normotensive conditions.[27,28] In PCA, the pressure at which the active diameter was significantly increased from the diameter at 75 mmHg was defined as the pressure of FD.

To detect effects of pregnancy or endotoxin infusion on myogenic tone, passive diameter and distensibility curves, Areas Under the Curves (AUC) were calculated using GraphPad Prism for Windows (Version 4) and the effect of pregnancy or endotoxin infusion was evaluated using two-way ANOVA.

Differences were considered significant at $P<0.05$. All data are expressed as mean ± SEM.

Results

Animal characteristics

Table 1 shows the characteristics of all animals. As expected, both pregnant groups had higher body weights compared to the nonpregnant group with the same treatment ($P<0.001$). Endotoxin infusion did not affect body weight in nonpregnant animals but slightly increased body weight in pregnant rats ($P<0.05$).

Table 1
Animal characteristics

	NP-CTL (n=9)	**NP-endotoxin** (n=9)	**P-CTL** (n=9)	**P-endotoxin** (n=10)
Body weight (g)	241.6 ± 6.6	247.2 ± 6.6	322.1 ± 7.0 *	345.8 ± 7.8 †‡
No. of pups	-	-	12.4 ± 1.0	13.2 ± 0.9
Length of pups (mm)	-	-	32.36 ± 0.22	31.57 ± 0.22 ‡

* P < 0.001 vs NP-CTL, † P<0.001 vs NP-endotoxin, ‡ P<0.05 vs P-CTL
NP-CTL, nonpregnant saline-treated; NP- endotoxin, nonpregnant endotoxin-treated; P-CTL, pregnant saline-treated; P-endotoxin, pregnant endotoxin-treated

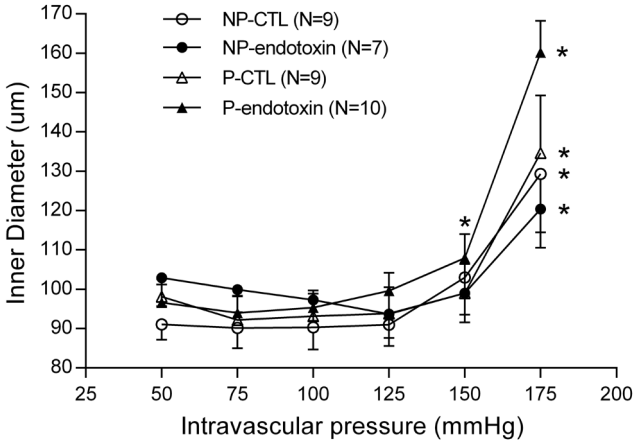

Figure 1A. Active diameter of posterior cerebral arteries at intravascular pressures from 50-175 mmHg. Pregnant endotoxin-treated rats (P-endotoxin; black triangles) underwent forced dilatation at lower intravascular pressures than the other groups, i.e. nonpregnant saline-treated rats (NP-CTL; open circles), nonpregnant endotoxin-treated rats (NP-endotoxin; black circles) and pregnant saline-treated rats (P-CTL; open triangles). * P<0.05 versus diameter at 75 mmHg from same group (repeated measures ANOVA)

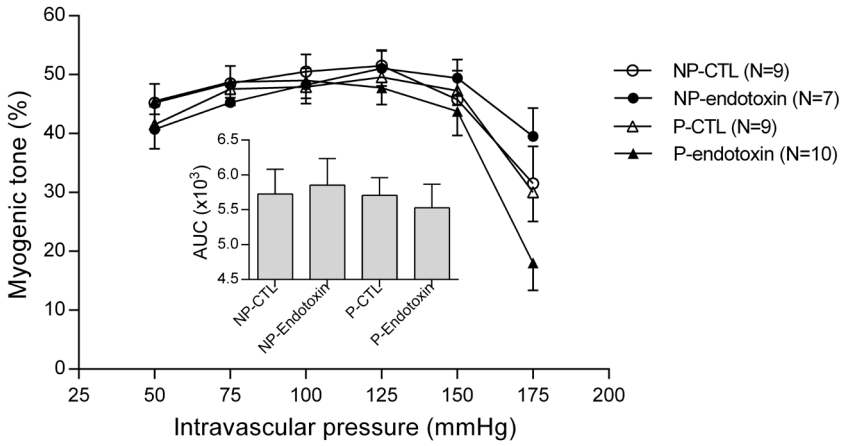

Figure 1B. Percentage myogenic tone of posterior cerebral arteries at intravascular pressures from 50-175 mmHg. Inserted bar graph: Area under the curve (AUC) of myogenic tone curves. Pregnancy or low-dose endotoxin treatment had no significant influence on myogenic tone. Nonpregnant saline-treated rats (NP-CTL; open circles), nonpregnant endotoxin-treated rats (NP-endotoxin; black circles), pregnant saline-treated rats (P-CTL; open triangles) and pregnant endotoxin-treated rats (P-endotoxin; black triangles).

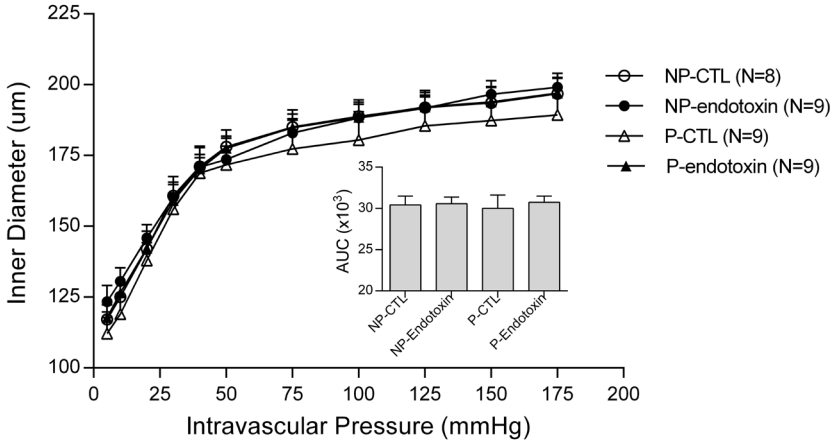

Figure 2A. Passive diameter of posterior cerebral arteries at intravascular pressures from 5-175 mmHg. Inserted bar graph: Area under the curve (AUC) of passive diameter curves. Pregnancy or low-dose endotoxin treatment had no significant influence on passive diameter. Nonpregnant saline-treated rats (NP-CTL; open circles), nonpregnant endotoxin-treated rats (NP-endotoxin; black circles), pregnant saline-treated rats (P-CTL; open triangles) and pregnant endotoxin-treated rats (P-endotoxin; black triangles).

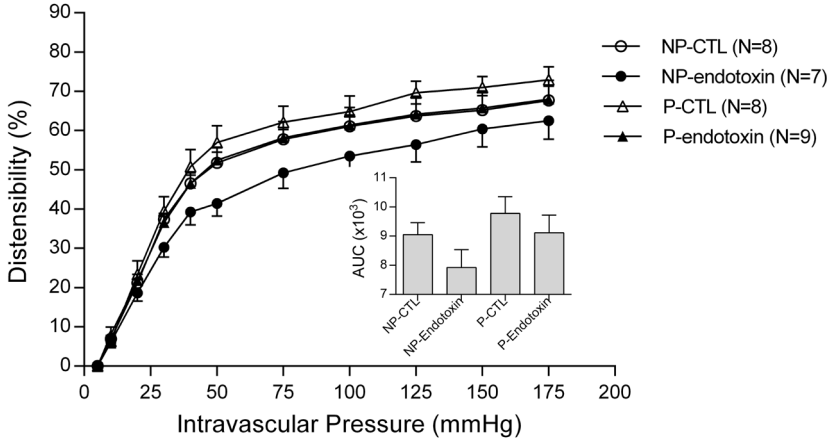

Figure 2B. Passive distensibility of posterior cerebral arteries at intravascular pressures from 5-175 mmHg. Inserted bar graph: Area under the curve (AUC) of passive distensibility curves. Pregnancy or low-dose endotoxin treatment had no significant influence on passive distensibility. Nonpregnant saline-treated rats (NP-CTL; open circles), nonpregnant endotoxin-treated rats (NP-endotoxin; black circles), pregnant saline-treated rats (P-CTL; open triangles) and pregnant endotoxin-treated rats (P-endotoxin; black triangles).

The number of pups and resorptions was similar for both pregnant groups. One animal of each group showed one resorption. However, pup length was significantly smaller in P-endotoxin compared to P-CTL rats ($P<0.05$).

PCA

Figure 1A shows the active diameter curves of PCA at intravascular pressures from 50 to 175 mmHg, which were used to assess the pressure of FD. All four groups underwent FD at the higher end of the intravascular pressure range. In nonpregnant animals and in P-CTL animal, FD occurred between 150-175 mmHg. However, in the P-endotoxin group, FD occurred between 125-150 mmHg.

Figure 1B shows the percent myogenic tone at different intravascular pressures for PCA. Stable myogenic tone (i.e. the slope was not different from 0) was observed in PCA in all groups at intravascular pressures from 50 to 150 mmHg (Table 2). To assess the influence of both pregnancy and endotoxin infusion on myogenic tone, AUC were compared using two-way ANOVA. There was no significant influence of either pregnancy or endotoxin on myogenic tone. However, as demonstrated in figure 1A, loss of myogenic tone occurred in all groups at the higher end of the intravascular pressure range.

To evaluate whether the differences in FD were due to differences in passive structure or diameter of PCA, we assessed the influence of both pregnancy and endotoxin infusion on passive diameters, wall thickness, wall-to-lumen ratio, wall stress and distensibility. For passive diameter and distensibility curves, AUC were compared between the groups using two-way ANOVA. This showed no significant effect of either pregnancy or endotoxin infusion on passive inner diameters of PCA (Figure 2A). Although NP-endotoxin animals appeared to have decreased distensibility compared to the other groups, no effect of either pregnancy or endotoxin was found (Figure 2B). Furthermore, passive wall thickness, wall-to-lumen ratio and wall stress were not significantly affected by either pregnancy or endotoxin infusion at any of the intravascular pressures from 5 to 175 mmHg. In table 3, passive wall thickness, wall-to-lumen ratio and wall stress are shown for the intravascular pressure of 75 mmHg, a pressure at which these vessels have been shown to operate under normotensive conditions.[28]

Table 2
Slopes of myogenic tone curves of posterior cerebral (50 - 150 mmHg) and mesenteric (40 - 140 mmHg) arteries

	NP-CTL (n=9 for PCA) (n=8 for MA)	**NP-endotoxin** (n=7 for PCA) (n=9 for MA)	**P-CTL** (n=9 for PCA and MA)	**P-endotoxin** (n=10 for PCA) (n=9 for MA)
Slope myogenic tone PCA	0.017 ± 0.013	0.093 ± 0.010	0.054 ± 0.011	-0.018 ± 0.010
Slope myogenic tone MA	0.216 ± 0.002 *	0.141 ± 0.011 *	0.116 ± 0.005 *	0.061 ± 0.004 *

* $P < 0.05$ vs zero
NP-CTL, nonpregnant saline-treated; NP-endotoxin, nonpregnant endotoxin-treated; P-CTL, pregnant saline-treated; P-endotoxin, pregnant endotoxin-treated

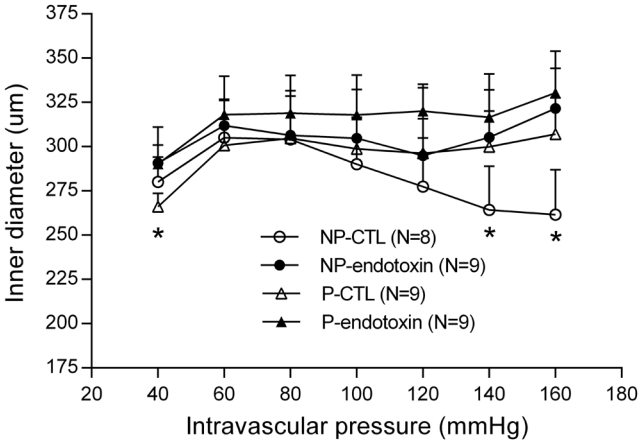

Figure 3A. Active diameter of mesenteric arteries at intravascular pressures from 40-160 mmHg. In nonpregnant saline-treated rats (NP-CTL; open circles) active diameter significantly decreased with increasing pressure. This was not observed in nonpregnant endotoxin-treated rats (NP-endotoxin; black circles), pregnant saline-treated rats (P-CTL; open triangles) and pregnant endotoxin-treated rats (P-endotoxin; black triangles). * P<0.05 versus diameter at 80 mmHg from same group (repeated measures ANOVA)

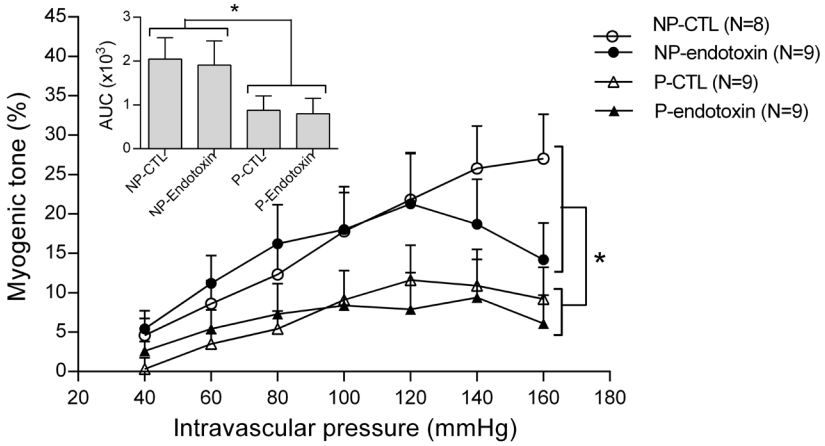

Figure 3B. Percentage myogenic tone of mesenteric arteries at intravascular pressures from 40-160 mmHg. Inserted bar graph: Area under the curve (AUC) of myogenic tone curves. Pregnancy decreased myogenic tone compared to nonpregnant animals at intravascular pressures from 120 to 160 mmHg. Nonpregnant saline-treated rats (NP-CTL; open circles), nonpregnant endotoxin-treated rats (NP-endotoxin; black circles), pregnant saline-treated rats (P-CTL; open triangles) and pregnant endotoxin-treated rats (P-endotoxin; black triangles).* P<0.05 for pregnant vs nonpregnant (two-way ANOVA of AUC)

MA

To investigate whether pregnancy and experimental preeclampsia influence the cerebral and systemic circulation similarly, simultaneous experiments were performed in MA.

In figure 3A the active diameter versus pressure curves are shown for all groups at intravascular pressures from 40 to 160 mmHg. Changes in active diameter at pressures below or above 80 mmHg were assessed using repeated measures ANOVA. In NP-CTL rats, as compared to 80 mmHg, active diameter significantly decreased with increasing pressures. This was not the case in the other three groups. Like in PCA, active diameter did not change below 80 mmHg in most groups, apart from P-CTL rats.

Figure 3B shows the level of myogenic tone of MA. Although MA from all groups developed myogenic tone, this appeared to be lower compared to PCA, which is in accordance with previous findings. Furthermore, in contrast to our findings in PCA, the slope of the myogenic tone curve of all groups of rats increased with increasing pressures between 40 and 140 mmHg (Table 2), indicating increasing myogenic tone over this pressure range. To assess the influence of both pregnancy and endotoxin infusion on myogenic tone, AUC were compared using two-way ANOVA. There was a significant effect of pregnancy on myogenic tone of MA with pregnant animals demonstrating decreased myogenic tone compared to nonpregnant animals ($P<0.05$). Endotoxin treatment did not affect myogenic tone in both nonpregnant and pregnant rats.

To assess whether decreased myogenic tone in MA during pregnancy was due to changes in passive diameter, passive pressure versus diameter curves of MA were evaluated (Figure 4A). As in PCA, AUC of passive inner diameters curves of MA were not significantly affected by pregnancy or endotoxin infusion at any of the intravascular pressures studied. Therefore, differences in myogenic tone could not be attributed to differences in passive diameter.

Table 3
Passive characteristics of posterior cerebral and mesenteric arteries

		NP-CTL (n=9 for PCA) (n=8 for MA)	**NP-endotoxin** (n=7 for PCA) (n=9 for MA)	**P-CTL** (n=9 for PCA and MA)	**P-endotoxin** (n=10 for PCA) (n=9 for MA)
PCA 75 mmHg	Wall thickness (um)	11.3 ± 1.0	10.6 ± 0.9	10.9 ± 0.7	10.6 ± 0.7
	Inner diameter (um)	185.0 ± 6.1	183.0 ± 5.0	177.3 ± 10.1	184.8 ± 4.8
	Wall-to-lumen ratio	0.061 ± 0.005	0.058 ± 0.004	0.064 ± 0.006	0.058 ± 0.005
	Wall stress (dynes/cm2 x 106)	0.859 ± 0.062	0.897 ± 0.066	0.840 ± 0.074	0.914 ± 0.069
MA 80 mmHg	Wall thickness (um)	21.6 ± 1.9	21.3 ± 2.3	20.1 ± 1.4	21.6 ± 1.2
	Inner diameter (um)	350.3 ± 13.7	358.8 ± 21.2	321.4 ± 10.5	349.9 ± 11.6
	Wall-to-lumen ratio	0.062 ± 0.005	0.059 ± 0.005	0.062 ± 0.004	0.062 ± 0.004
	Wall stress (dynes/cm2 x 106)	0.912 ± 0.090	0.949 ± 0.070	0.889 ± 0.070	0.882 ± 0.044

NP-CTL, nonpregnant saline-treated; NP-endotoxin, nonpregnant endotoxin-treated; P-CTL, pregnant saline-treated; P-endotoxin, pregnant endotoxin-treated

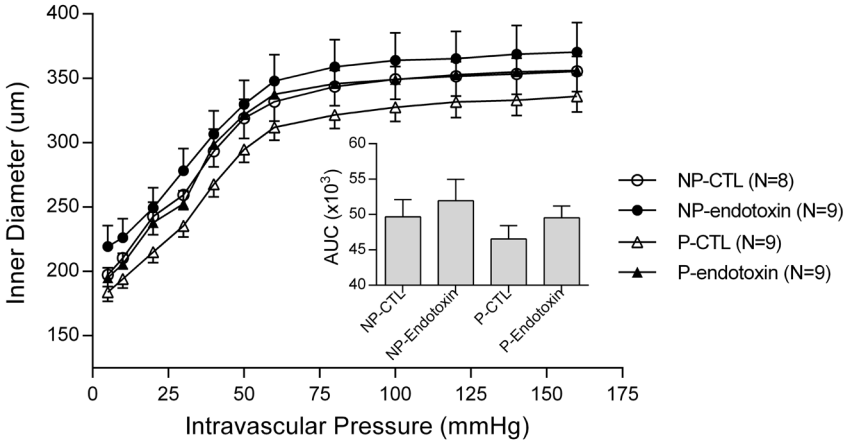

Figure 4A. Passive diameter of mesenteric arteries at intravascular pressures from 5-160 mmHg. Inserted bar graph: Area under the curve (AUC) of passive diameter curves. Pregnancy or low-dose endotoxin treatment had no significant influence on passive diameter. Nonpregnant saline-treated rats (NP-CTL; open circles), nonpregnant endotoxin-treated rats (NP-endotoxin; black circles), pregnant saline-treated rats (P-CTL; open triangles) and pregnant endotoxin-treated rats (P-endotoxin; black triangles).

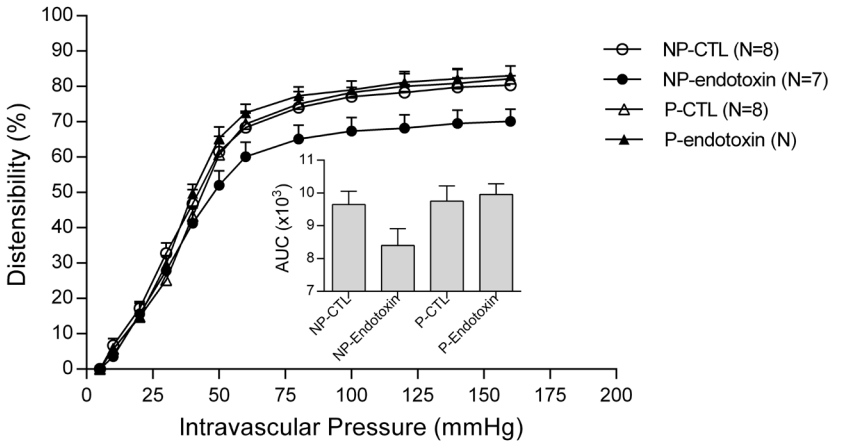

Figure 4B. Passive distensibility of mesenteric arteries at intravascular pressures from 5-160 mmHg. Inserted bar graph: Area under the curve (AUC) of passive distensibility curves. Pregnancy or low-dose endotoxin treatment had no significant influence on passive distensibility. Nonpregnant saline-treated rats (NP-CTL; open circles), nonpregnant endotoxin-treated rats (NP-endotoxin; black circles), pregnant saline-treated rats (P-CTL; open triangles) and pregnant endotoxin-treated rats (P-endotoxin; black triangles).

Furthermore, wall thickness, wall-to-lumen ratio and wall stress were evaluated in relaxed vessels and were not affected by either pregnancy or endotoxin infusion any of the intravascular pressures studied. In table 3, these measurements are shown for the intravascular pressure of 80 mmHg, a pressure to which these vessels are exposed under normotensive conditions.[27] As in PCA, percent distensibility of MA tended to be decreased in NP-endotoxin animals compared to the other groups, however, no significant differences in AUC of distensibility curves were found (Figure 4B).

Discussion

In the present study, we investigated the effect of pregnancy and low-dose endotoxin infusion, which is an established model for preeclampsia, on function and structure of Wistar rat PCA and MA. In PCA, we found that while normal pregnancy did not affect structure or function, low-dose endotoxin infusion decreased the pressure at which FD occurred compared to both normal pregnant and nonpregnant rats. Within the autoregulatory pressure range, myogenic tone was not affected by either pregnancy or low-dose endotoxin infusion. Our findings suggest that low-dose endotoxin infusion during pregnancy, and not pregnancy alone, may predispose the brain to autoregulatory breakthrough and edema formation when blood pressure is increased as seen in eclampsia. In MA, structure was unaffected by pregnancy or low-dose endotoxin. However, in contrast to PCA, pregnancy alone decreased myogenic tone of MA, with no additional effect of low-dose endotoxin infusion.

Prior studies evaluating the influence of pregnancy on the cerebrovasculature are relatively scarce and somewhat contradictory. In vitro studies by Cipolla et al showed diminished myogenic reactivity[14] and earlier FD[13] of PCA in late-pregnant versus nonpregnant Dahl salt-sensitive and Sprague-Dawley rats, respectively, suggesting that pregnancy alone would predispose to loss of autoregulation. However, others found no influence of pregnancy on myogenic reactivity of a different cerebral blood vessel (the middle cerebral artery) in Sprague-Dawley rats.[29] Furthermore, an in vivo study showed autoregulatory breakthrough at similar pressures in late-pregnant and nonpregnant Sprague-Dawley rats.[30] We used in vitro techniques comparable to those used by Cipolla et al and found no effect of pregnancy alone on PCA myogenic tone or pressure of FD. This difference might be explained by use of different rat strains as differences in mesenteric artery function during pregnancy have been described for Wistar versus Sprague-Dawley rats.[31] Whether such inter-strain differences may also occur in cerebral arteries is currently unkown. Despite the fact that we found no effect of pregnancy on myogenic tone or FD in PCA, earlier FD was observed after low-dose endotoxin infusion in pregnant Wistar rats. Knowledge about effects of experimental preeclampsia on cerebral arteries is even more limited. Two studies using models of hypertension in pregnancy showed no differences in myogenic reactivity[14] and pressure of FD[15] in PCA between normo- and hypertensive pregnant Dahl salt-sensitive and

Sprague-Dawley rats, respectively. However, middle cerebral arteries from experimental preeclamptic Sprague-Dawley rats, induced by reduced uterine perfusion, have decreased myogenic reactivity and increased brain water content compared to normal pregnant rats.[29] In line with this study, our data also suggest that low-dose endotoxin infusion during pregnancy impairs cerebral autoregulation and thus may sensitise the brain for edema development and eclampsia.

Earlier FD of PCA in preeclamptic rats appeared not to be due to structural changes of these arteries since neither pregnancy nor low-dose endotoxin did affect passive characteristics of PCA. These findings suggest the absence of pregnancy or experimental preeclampsia induced structural remodeling of PCA. It is well known that cerebral arteries from nonpregnant animals undergo structural remodeling in response to hypertension. This is thought to protect the downstream microcirculation from increased hydrostatic pressure.[32,33] However, studies in two rat models of hypertensive pregnancies have shown that pregnancy prevented this remodeling in PCA[14,15] and even reversed already established remodeling.[34] The lack of remodeling in preeclamptic rat PCA in our study is in line with these earlier findings. Unfortunately, for various technical reasons it was not possible to measure blood pressure in the rats in our study. However, we expect a mild increase in blood pressure, mainly in the last few days of pregnancy.[18-20,26] The lack of remodeling could also be due to this relatively mild hypertension, only at the end of pregnancy or to the short duration (one week) of hypertension. The mechanistic basis for forced dilatation at lower blood pressure in PCA from preeclamptic Wistar rats remains therefore unclear, but might be related to increased sensitivity of cerebral arteries to nitric oxide (NO). Recently, it was shown that low-dose endotoxin-treated Wistar pregnant rats showed enhanced sensitivity of PCA to the dilatory effect of the NO donor NONOate.[16] In addition, expression of vascular inducible NO synthase (iNOS) was augmented in these animals[16], which might increase NO levels. Whether similar changes occurred in the rats in our study remains to be studied, as the model of Cipolla et al, is different from our model in several aspects. Major differences are infusion of a 1.5 times higher dose of endotoxin and infusion under isoflurane anesthesia, rather than under awake conditions.[16] These differences may have affected the model, since our lab has previously demonstrated that infusion of endotoxin into pregnant animals under anesthesia did not result in hypertension (unpublished data). Additionally, the time frame between endotoxin infusion and the experiments was different; in the Cipolla study, rats were sacrificed 3 or 2 days prior to delivery, while in our study the rats were sacrificed 1 day prior to delivery.[16]

To investigate whether pregnancy and low-dose endotoxin infusion similarly affect cerebral and peripheral resistance arteries, function and structure of MA were assessed. In contrast to PCA, pregnancy, regardless of infusion of low-dose endotoxin, appeared to decrease myogenic tone of MA due to a different response of active diameter to increasing intravascular pressure. Whereas, similar to other studies,[35,36] MA active diameter in NP-

CTL rats decreased in response to increasing pressure, they were relatively constant in pregnant animals. Our finding of decreased myogenic tone in MA of Wistar rats during pregnancy is not surprising in the face of the hemodynamic characteristics of normal pregnancy. Decreased myogenic tone may be one of the mechanisms underlying the decreased peripheral vascular resistance seen during pregnancy.[37,38] However, earlier findings on myogenic behaviour of MA during pregnancy appear contradictory. While some studies have shown a similar decrease in myogenic tone in pregnant versus nonpregnant rats and mice,[7,35] others did not.[10,39] Furthermore, while we did not see an effect of low-dose endotoxin on myogenic tone, others have found increased myogenic reactivity in a model for preeclampsia induced by reduced uterine perfusion pressure in pregnant Sprague-Dawley rats.[11,40] These differences might be due to differences in rat strain, preeclampsia rat model or experimental techniques.

Similar to PCA, no effect of either pregnancy or low-dose endotoxin infusion was found on structural properties of MA suggesting that MA, like PCA, did not undergo structural remodeling. In addition, distensibility was not affected, suggesting no change in wall components.[41] Only few studies have examined the influence of pregnancy on structural properties of MA. In contrast to our findings, they found increased distensibility[41,42] and decreased wall thickness during pregnancy.[42] However, both studies were performed in Sprague-Dawley rats, a different rat strain than used in our study. We also measured angiotensin II sensitivity and endothelial function in the aortas of these endotoxin infused pregnant rats and found increased angiotensin II sensitivity and endothelial dysfunction in the aortas of the pregnant endotoxin infused rats compared with control pregnant rats.[23] This suggests that, although we found no effect of endotoxin in pregnant animals on MA in the present study, peripheral vascular changes are apparent in these rats.

In conclusion, our study for the first time showed that low-dose endotoxin infusion, but not pregnancy alone, induced earlier FD in PCA. This could not be explained by structural remodeling of PCA. This earlier FD may predispose the brain to autoregulatory breakthrough and edema formation during states of increased blood pressure (e.g. eclampsia). In addition, myogenic tone of MA and PCA appeared to be differentially affected by pregnancy and low-dose endotoxin infusion. While pregnancy decreased myogenic tone of MA, independently of low-dose endotoxin infusion, myogenic tone of PCA was not affected. This might be due to functional difference between the mesenteric and cerebral vascular bed. Whereas the mesenteric vascular bed may show decreased myogenic tone in the light of the decreased systemic peripheral vascular resistance during pregnancy,[37,38] the cerebral vascular system has to exert a tight autoregulatory blood flow control to prevent ischemia or hyperperfusion leading to blood-brain barrier disruption.[43,44]

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5

S100B BRAIN EXPRESSION AND PLASMA CONCENTRATIONS IN A PREECLAMPSIA RAT MODEL

Pregnancy Hypertens. 2011 Apr;1(2):143–149

Abstract

Objective

To assess brain damage using the neuroinflammation marker S100B in a preeclampsia rat model.

Methods

Non-pregnant and pregnant rats were infused with saline or low-dose endotoxin on day 14 of pregnancy. S100B expression in the brain (immunohistochemistry) and S100B plasma concentrations (ELISA) were studied.

Results

No differences in S100B expression in brain tissue were observed between the four groups. Pregnant endotoxin-treated animals did not show increased levels of plasma S100B levels as compared with control pregnant rats, while significantly higher plasma S100B levels were found in non-pregnant endotoxin versus pregnant endotoxin infused rats.

Conclusion

Pregnancy nor experimental preeclampsia, alter S100B in rat brain, or in plasma. Increased plasma S100B in non-pregnant endotoxin-treated rats may indicate brain injury in these rats, whereas pregnancy might be protective.

Introduction

Pre-eclampsia is responsible for the world's largest maternal mortality rates, mostly due to acute cerebral complications such as eclampsia (posterior reversible encephalopathy syndrome (PRES)), and intracerebral hemorrhage.[1] PRES is thought to be caused by a failure of the brain's autoregulatory response to increases in blood pressure in conjunction with endothelial cell dysfunction. It is associated with a loss of integrity of the blood–brain barrier; inflammatory cells and fluid can penetrate the brain and cause edema and cell death. Interruption of this delicate balance between capillary and cellular perfusion pressures may lead to the neurological complications of preeclampsia.[1]

S100B is an acidic calcium-binding protein, mainly expressed by astrocytes, oligodendrocytes and Schwann cells.[2] Experimental and clinical studies have shown S100B to be involved in neuroinflammation.[3] Because approximately 95% of S100B is located in the CNS, the results of several studies, both human and animal, have suggested that an increase in S100B levels in blood as well as increased expression in brain tissue could be a potential marker of neuronal injury indicating astrocytic death, reactive gliosis and/or blood–brain barrier dysfunction.[4-7]

Neuroinflammation is a key component of various central nervous system (CNS) diseases, promoting both reparation and damage of neural tissue.[3] The use of neuroinflammatory biomarkers (such as S100B) in hypertensive disease in pregnancy may represent a novel avenue for early diagnosis, and eventually prevention, of cerebrovascular complications in preeclamptic women who present with neurological signs and symptoms, but current data are limited.[5-8]

To assess the possible relationship between preeclampsia and neuronal brain damage, we investigated the brain injury marker S100B in brain tissue and plasma in a rat model for mild preeclampsia.

Materials and methods

Animals

Female Wistar outbred rats (200–220 g) were housed in standard conditions, in a temperature- and light-controlled room (12 h light–dark cycle), with free access to water and food. Vaginal smears were taken daily, and rats were rendered pregnant by housing them on proestrus with fertile male Wistar rats for one night. When spermatozoa were detected in the smear the next day, this day was designated day 0 of pregnancy. A permanent cannula was inserted in the right jugular vein in all rats under 2% isoflurane/oxygen anesthesia according to standard methods.[9] The cannula allows stress-free infusions.

Experimental design

The ultra-low-dose endotoxin rat model for preeclampsia was used to simulate a preeclamptic state.[10] This model is characterized by hypertension and proteinuria in the last week of pregnancy. Endotoxin, derived from *Escherichia coli* (*E. coli*, 0.55:B5, Whittaker MA Bioproducts, Walkerville, MD) was dissolved at a dose of 1.0 µg/kg body weight in 2 ml of pyrogen free saline solution. Endotoxin (or saline for control) was infused via the jugular vein cannula with an infusion rate of 2.0 ml/h for the duration of 1 h. Pregnant rats were infused at day 14 of pregnancy, nonpregnant rats 6 days prior to sacrifice.

The rats were divided into four groups: (1) nonpregnant rats infused with saline (nonpregnant controls, n=5); (2) nonpregnant rats infused with low-dose endotoxin (n=7); (3) pregnant rats infused with saline (pregnant controls, n=7); and (4) pregnant rats infused with low-dose endotoxin (n=7).

Because we were not able to obtain blood samples in all rats, the study population with regard to the ELISA consisted of: (1) nonpregnant rats infused with saline (n=5); (2) nonpregnant rats infused with low-dose endotoxin (n=5); (3) pregnant rats infused with saline (n=7); and (4) pregnant rats infused with low-dose endotoxin (n=6).

Preparation of brain tissue

At day 20 of pregnancy or 6 days after infusion in nonpregnant rats, rats were decapitated under isoflurane/oxygen anesthesia. Immediately after decapitation, trunk blood of the rat was collected in heparin tubes. Blood samples were centrifuged (562g, 10 min), blood plasma collected and centrifuged again (1083g, 10 min). Plasma was stored at -80 °C until analysis.

The brain was quickly removed and divided into forebrain, cerebellum and brain stem. Brain tissue was fixed in 4% paraformaldehyde for 24 h and, until embedding, stored in 70% ethanol. Before embedding in paraffin wax, tissue dehydrated by submitting to ethanol (96% and 100%) and finally xylol. Of the forebrain, cerebellum and brain stem, sections of 5 lm were cut and mounted on silane coated glass slides (Starforst adhesive grün, Knittel Gläser, Braunschweig, Germany).

Immunohistochemical staining of S100B in brain tissue

For immunohistochemical analysis, paraffin embedded sections were dewaxed in xylene and rehydrated in ethanol. S100B antigen was retrieved by heating sections in a trisaminomethane/ethylenediaminetetraacetic acid (Tris/EDTA) buffer (10 mM Tris/1 mM EDTA, pH 9.0) in a microwave oven (400 W) for 15 min. Sections were cooled in Tris/EDTA for 30 min and then washed in phosphate buffered saline (PBS) for 5 min. After incubation with normal rabbit serum (Dako, Glostrup, Denmark, 1:10, 30 min, room temperature (RT)),

the sections were incubated with the primary antibody (polyclonal rabbit anti-S100B (Dako, Glostrup, Denmark), 1:200, 60 min, RT). Endogenous peroxidase activity was blocked using 0.075% hydrogen peroxide (H_2O_2) in PBS (30 min, RT). After incubation with a secondary antibody (swine anti-rabbit horseradish peroxidase labeled (Dako, Glostrup, Denmark, 1:100, 30 min, RT)), the staining was finished by performing an 3-amino-9-ethyl carbazole (AEC)-staining (10 min, RT). Sections were counterstained with haematoxylin.

Of each section of the forebrain, cerebellum and brain stem, 10 comparable fields were photographed with a computerized microscope (Leica DFC420C, Wetzlar, Germany) at a 400x magnification. Each photograph covers 0.06097 mm^2. Of each section, the fields are comparable with fields in other sections with regard to anatomical location and estimated total number of cells. The hippocampus, a part of the forebrain, was also photographed. Because the hippocampus is a small structure, only five fields were photographed to avoid overlap. The hippocampus was not present in all sections: sections obtained in the lateral part of the forebrain did not contain hippocampal tissue. Hippocampal tissue was not present in one nonpregnant rat infused with saline, two nonpregnant rats infused with low-dose endotoxin, two pregnant rats infused with saline and two pregnant rats infused with low-dose endotoxin. Of each photograph, the total number of S100B-positive cells was scored blindly by two examiners. The average of the two examiners per photograph was calculated. The number of positive cells of all photographs of each section was added up, and expressed as number of positive cells/mm^2. The fields photographed were compared with fields in the same brain region from other rats for differences in distribution of S100B-positive cells and intensity of the S100B-staining.

ELISA to determine S100B concentrations in plasma

To determine the S100B concentration in rat plasma, we used a commercial available enzyme-linked immunosorbent assay (ELISA) kit (Biovendor, Heidelberg, Germany). All the procedures were performed according to the manufacturer's protocol. All samples were measured in duplicates.

Statistical analysis

Statistical analysis was performed using SPSS 16.0 (SPSS Inc., Chicago, USA). All results were expressed as mean ± standard error of mean (SEM). The effect of pregnancy or endotoxin was evaluated using Two-way ANOVA and post hoc Bonferroni. $P<0.05$ was considered significant.

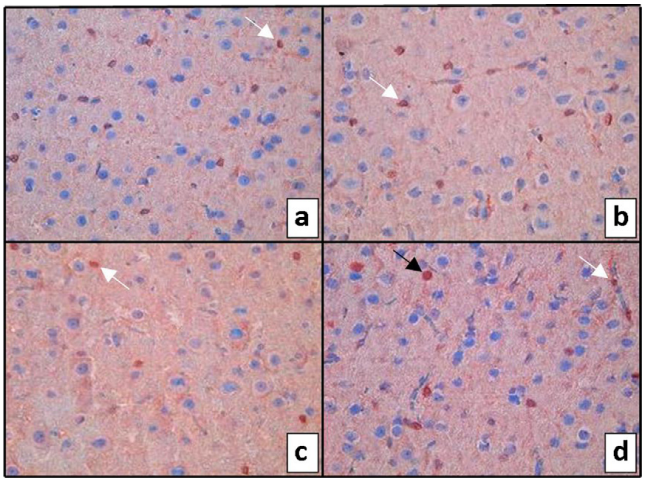

Figure 1. Representative microphotographs of the forebrain immunostained for S100B and counterstained with haematoxylin of nonpregnant and pregnant rats infused with saline or endotoxin. **(a)** Nonpregnant rat infused with saline, **(b)** pregnant rat infused with saline, **(c)** nonpregnant rat infused with endotoxin and **(d)** pregnant rat infused with endotoxin. In the forebrain, the S100B protein seems to be mainly localized in the glial cells (white arrow), but some neurons (black arrow) also contain S100B. S100B-positive cells are indicated by arrows: white arrow, glial cell; black arrow, neuronal cell. Magnification: 400x.

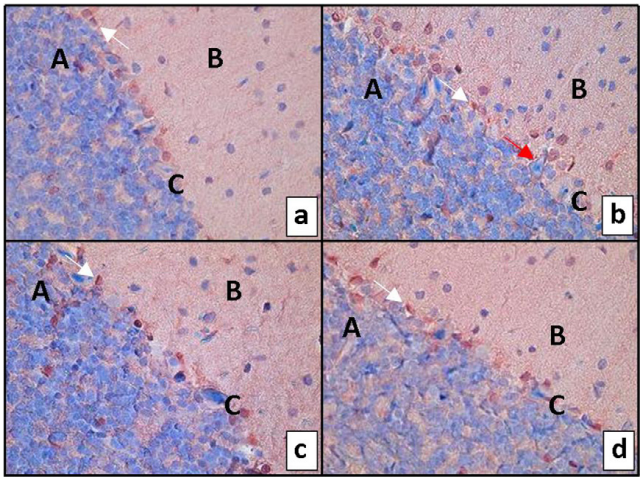

Figure 2. Representative microphotographs of the cerebellum immunostained for S100B and counterstained with haematoxylin of nonpregnant and pregnant rats infused with saline or endotoxin. **(a)** Nonpregnant rat infused with saline, **(b)** pregnant rat infused with saline, **(c)** nonpregnant rat infused with endotoxin and **(d)** pregnant rat infused with endotoxin. Different types of layers can be observed in these microphotographs: the granule cell layer (A) and the molecular layer (B) of the cerebellum. Between these two layers a layer of Purkinje cells (C) can be observed. S00B positive glial cells (white arrows) are mainly localized in the Purkinje cell layer; Purkinje cells (red arrow) are negative for S100B. Magnification: 400x.

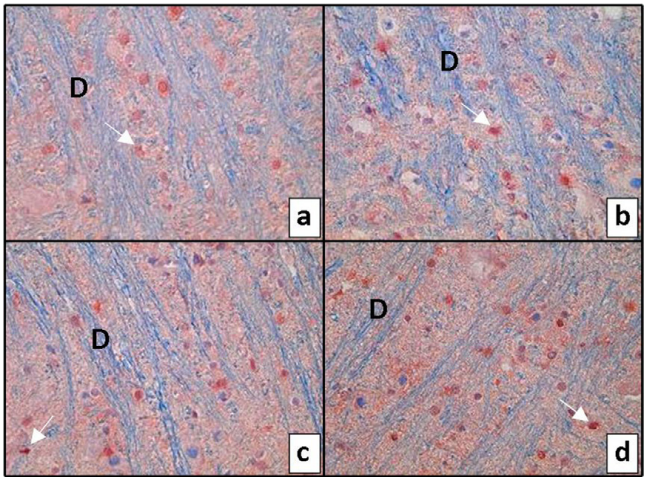

Figure 3. Representative microphotographs of the brain stem immunostained for S100B and counterstained with haematoxylin of nonpregnant and pregnant rats infused with saline or endotoxin. **(a)** Nonpregnant rat infused with saline, **(b)** pregnant rat infused with saline, **(c)** nonpregnant rat infused with endotoxin and **(d)** pregnant rat infused with endotoxin. In the brain stem, nerve tracts can be observed (D), surrounded by glial cells. S100B-positive glial cells are indicated by white arrows. Magnification: 400x.

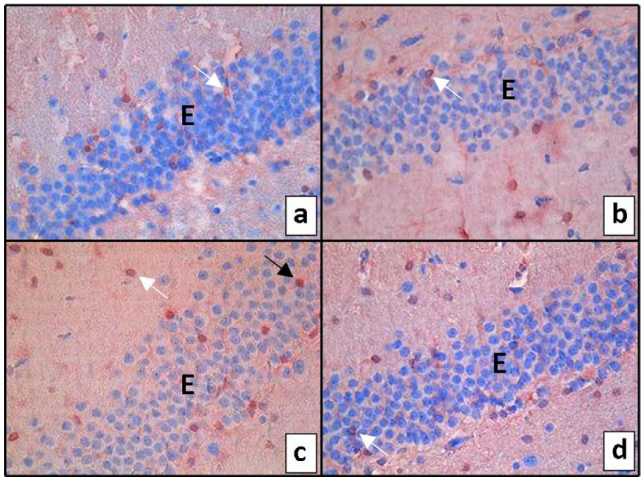

Figure 4. Representative microphotographs of hippocampus immunostained for S100B and counterstained with haematoxylin of nonpregnant and pregnant rats infused with saline or endotoxin. **(a)** Nonpregnant rat infused with saline, **(b)** pregnant rat infused with saline, **(c)** nonpregnant rat infused with endotoxin and **(d)** pregnant rat infused with endotoxin. The dentate gyrus is shown in these photographs (E). The dentate gyrus is surrounded by both glial cells and neurons. S100B-positive cells are indicated by arrows: white arrow, glial cell; black arrow, neuronal cell. Magnification: 400x.

Results

S100B expression in brain tissue

To examine the expression of the S100B protein in brain tissue, we performed immunohistochemical staining of sections of the forebrain, cerebellum and brain stem.

Figs. 1–4 demonstrate representative microphotographs of the forebrain, cerebellum, brain stem and hippocampus, immunostained for the S100B protein.

In the forebrain, the S100B protein seems to be mainly localized in the glial cells, but some neurons also contain S100B. In the forebrain, no major differences between the four groups of rats with regard to the intensity of the S100B-staining were observed (Fig. 1). Moreover, in all groups, the S100B-positive cells were diffusely distributed throughout the sections, and not grouped.

In Fig. 2, representative microphotographs of the cerebellum are shown. In all four groups, S100B-positive glial cells are mainly localized in the layer between the granule cell layer (A) and the molecular layer (B) of the cerebellum, which is called the Purkinje cell layer (C). The Purkinje cells are negative for the S100B protein. No major differences were observed with regard to the intensity of the S100B-staining in the cerebellum between the different groups.

Fig. 3 shows representative microphotographs of the brain stem. In these photographs, nerve tracts (D) can be observed. S100B-positive cells in the brain stem are mainly glial cells. With regard to the intensity of the staining of the sections of the brain stem, no major differences between the four groups could be observed. No difference in distribution of S100B-positive cells was observed.

Representative microphotographs of the hippocampus are demonstrated in Fig. 4. The dentate gyrus (E) is shown in these photographs. Both neurons and glial cells contain S100B. No major differences between the four groups with regard to the intensity of the S100B-staining in the hippocampus could be observed. There were no differences with regard to the distribution of the S100B-positive cells between the four different groups in the hippocampus.

Table 1 demonstrates the number of S100B positive cells per mm^2 in the different brain regions. No significant differences were detected in the number of S100B positive cells in the different brain regions between the nonpregnant controls and the pregnant controls, nor between the pregnant controls and the pregnant endotoxin group. Differences in nonpregnant endotoxin versus pregnant endotoxin and nonpregnant controls versus nonpregnant endotoxin were not observed either.

S100B concentration in plasma

No significant differences were observed between the nonpregnant controls and the

pregnant controls, nor between the pregnant controls and the pregnant endotoxin group (Fig. 5). In the nonpregnant endotoxin group, the S100B concentration in plasma was significantly higher when compared with the pregnant endotoxin group ($P<0.05$).

Discussion

This study demonstrates the expression of the S100B protein in different brain regions and plasma S100B concentrations in a preeclampsia rat model. No differences in S100B brain expression nor plasma concentrations were observed between pregnant and nonpregnant controls. Pregnancy per se, thus, does not influence the expression of the S100B protein in the rat brain nor S100B plasma concentrations. Moreover, no differences in S100B plasma concentrations nor S100B brain expression were detected between the pregnant controls and pregnant rats with experimental preeclampsia. Thus, in this mild preeclampsia rat model, we were unable to demonstrate evidence of brain injury. However, the S100B concentration in plasma was significantly higher in the nonpregnant endotoxin treated rats, when compared with the pregnant endotoxin treated rats (preeclampsia rat model). Pregnancy might have a protective effect on brain tissue after endotoxin infusion in rats.

In this study, we used the ultra-low-dose endotoxin preeclampsia rat model, which has been used for several years in preeclampsia research.[10-14] The histopathological and clinical events in this model simulate the predominant features of mild human preeclampsia such as a significant increase in blood pressure and in urinary albumin excretion.[10] The fact that we did not find evidence of brain injury in this model, may suggest that this mild preeclampsia rat model is not severe enough to induce cerebrovascular pathology. This may be in line with the fact that pregnant women with severe preeclampsia are more at risk for developing cerebrovascular pathology (PRES) when compared with women with mild preeclampsia.[1] Therefore, it is possible that S100B plasma concentrations and brain expression are increased in animal models for severe preeclampsia, when compared with animal models for mild preeclampsia.

To our surprise, we observed increased concentrations of S100B in the plasma of nonpregnant endotoxin treated animals. In contrast, we did not find increased expression

Table 1
Number of S100B positive cells per mm^2 in the forebrain, cerebellum, brain stem and hippocampus in different groups of rats.

Group	Brain regions (mean ± SEM)			
	Forebrain	Cerebellum	Brain stem	Hippocampus
Nonpregnant saline	152.8 ± 11.6 (n=5)	182.9 ± 12.5 (n=5)	242.9 ± 46.1 (n=5)	192.9 ± 20.0 (n=4)
Nonpregnant endotoxin	151.1 ± 15.4 (n=7)	220.2 ± 17.1 (n=7)	300.8 ± 51.9 (n=7)	224.1 ± 20.4 (n=5)
Pregnant saline	165.6 ± 13.5 (n=7)	238.2 ± 20.0 (n=7)	260.9 ± 21.3 (n=7)	221.6 ± 8.5 (n=5)
Pregnant endotoxin	170.2 ± 12.7 (n=7)	191.3 ± 12.2 (n=7)	273.4 ± 35.7 (n=7)	211.6 ± 8.7 (n=5)

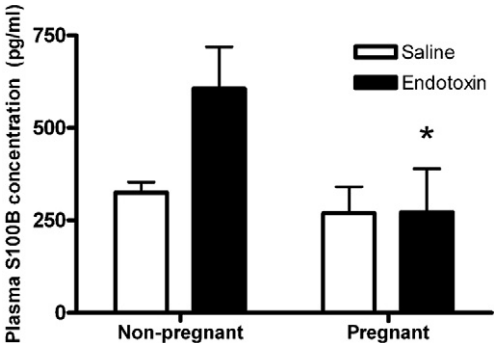

Figure 5. Mean (±SEM) of S100B concentrations in plasma of nonpregnant (left set of bars) and pregnant rats (right set of bars) after infusion of saline (open bars) or endotoxin (black bars). *P<0.05 vs nonpregnant rats infused with endotoxin, Two-way ANOVA (P=0.0472) followed by post hoc Bonferroni.

of S100B in brain tissue of these animals. Our finding of increased plasma S100B is also in contrast to previous studies, in which we observed no effect of endotoxin in nonpregnant rats on various parameters.[10-12] It may, however, be in line with several studies reporting neuroinflammation induced by endotoxin in rats.[15-16] The dose of endotoxin used in our study, however, is much lower and given intravenously rather then intraperitoneally or via intracerebral injection. The high S100B plasma concentrations in the nonpregnant endotoxin-treated rats might thus be caused by endotoxin-induced neuroinflammation.

Little is known about S100B levels and their significance in the peripheral blood, and cerebrospinal fluid in human (pre)eclampsia. Schmidt et al. studied serum S100B concentrations in women with several forms and severity of hypertensive disease in pregnancy.[5] This small study included 50 women. Of these women, 10 had experienced eclampsia, and 18 women had preeclampsia. No significant difference in serum S100B concentration could be demonstrated between the preeclamptic group and the normotensive group but serum S100B was significantly higher in eclampsia. Unfortunately, hardly any clinical characteristics of the study population with preeclampsia were provided, and therefore, no conclusions can be drawn with regard to the clinical relevance and utility of S100B serum levels. Whether the increased levels of plasma S100B only arise from the CNS in eclampsia remains to be established, since in pregnancy, S100B may also be found in fetal tissue. Tskitishvili et al. found the percentage of positively stained amniotic epithelial cells increased in preeclampsia compared with healthy pregnant controls, while in the amniotic fluid the S100B concentration was significantly higher in patients with preeclampsia, when compared with the healthy controls.[8]

Although little is known about S100B in preeclampsia, it has the potential to become an important clinical biomarker of central nervous system injury and neuroinflammation in pregnancy and preeclampsia.[17] In various central nervous system disorder, such as

subarachnoid hemorrhage, ischemic stroke and traumatic injury, the level of the S100B protein in peripheral blood samples is elevated.[17] The expression of the S100B protein in brain tissue and S100B concentrations in peripheral blood have also been investigated in several animal models for brain injury. S100B expression in the stroke-prone hypertension rat model was increased compared with healthy, normotensive rats.[6] Hippocampal S100B expression was increased in rats exposed to chronic cerebral hypoperfusion when compared with control rats.[18] Lipcsey et al. demonstrated increased expression of the S100B protein in the pig brain, mainly in the astrocytes, in septic encephalopathy.[19] Tanaka et al. demonstrated significant increases in serum S100B levels in a rat with cerebral hemorrhage when compared with rats without cerebral hemorrhage.[20] These nonpregnant animal models demonstrate that brain injury (e.g. stroke and hypoperfusion) can cause a higher S100B expression in rat brain tissue and higher S100B concentrations in peripheral blood. Whether also in pregnancy S100B will be affected by brain injury, remains to be established.

Concluding, this study demonstrates that pregnancy nor mild preeclampsia influenced the expression of the S100B protein in brain tissue and S100B plasma concentrations in the endotoxin rat model. Taking into account that the pathophysiology of PRES in (pre)eclampsia involves breakthrough of the blood–brain barrier and, eventually, ischemic damage to cells in the central nervous system, it is feasible that an increase in the S100B protein could be observed in preeclamptic women with impending PRES. To further elucidate the clinical utility of brain neuroinflammatory biomarkers animal studies with models for severe preeclampsia should be performed.

Acknowledgment
We would like to thank Prof. Dr. E.A. van der Zee for his help.

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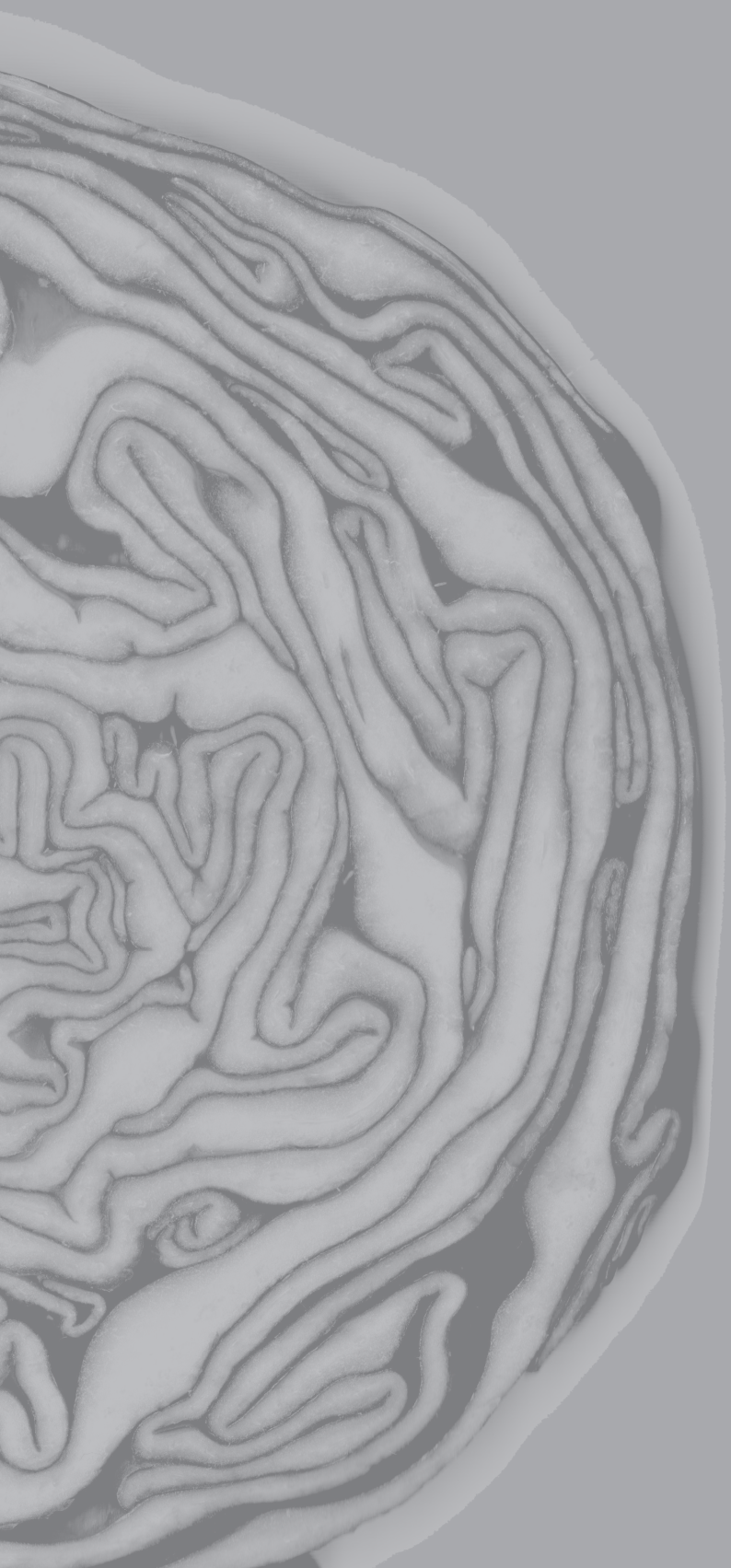

PART 2

HUMAN STUDIES

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6

VISUAL DISTURBANCES IN PRE(ECLAMPSIA)

Obstet Gynecol Surv. 2012 Apr;67(4):242-50

Abstract

This review aims to summarize existing information concerning visual disturbances in (pre)eclampsia that have been described in the literature. Preeclampsia is one of the leading causes of maternal and fetal morbidity and mortality worldwide. Visual disturbances in (pre)eclampsia seem to be frequent phenomena. Therefore, the obstetrician/gynecologist may encounter women with serious, and sometimes debilitating, pathology of the visual pathways. Established ophthalmic entities associated with (pre)eclampsia are cortical blindness, serous retinal detachment, Purtscher-like retinopathy, central retinal vein occlusions, and retinal or vitreous hemorrhages. Ensuing visual symptoms include blurry vision, diplopia, amaurosis fugax, photopsia, and scotomata, including homonymous hemianopsia. In general, aside from lowering the blood pressure and preventing (further) seizures with magnesium sulfate, no specific therapy seems indicated for (pre)eclamptic women who experience visual changes. Although in most cases visual acuity returns to normal within weeks to months after the onset of symptoms, rarely permanent visual impairment can occur. Health care providers such as emergency room physicians, obstetricians, family physicians, neurologists, and ophthalmologists should be aware that acute onset of visual symptoms in pregnant women can be the first sign of (pre)eclampsia. Given that visual changes are a diagnostic criterion for severe preeclampsia, obstetricians should appreciate the significance of these changes and discuss appropriate diagnostic options with the ophthalmologist. Affected women can be reassured that most cases are transient.

Vignette

A 24-year-old primigravid woman at 37 weeks' gestation was brought to the emergency department by her family. The family stated that the woman had woken up that morning unable to see. The woman herself denied any visual disturbance but did complain of nausea and frontal headache. Her pregnancy had been uneventful before this time. On examination, she appeared confused and disoriented as to time. Her blood pressure was 165/110 mm Hg, heart rate 128 beats per minute, and she had slight edema in her hands and feet. Neurologic and ophthalmic examination revealed no abnormalities, except hyperreflexia and severe impairment of vision, although the woman continued to disagree that she had visual impairment. Pupillary reflexes were intact and the motility undisturbed. Fundoscopic examination showed no abnormalities of the optic disc or macula. Laboratory testing revealed 3+ proteinuria and elevated uric acid. A T2-weighted magnetic resonance imaging (MRI) scan demonstrated bilateral hyperintense signals in the parieto-occipital lobes. Based on the signs and symptoms described earlier, the diagnosis made was preeclampsia complicated by cortical blindness.

Introduction

Pregnancy can affect multiple organs, including the eyes. For example, the pregnant state is associated with increased corneal thickness[1,2] and curvature,[3] and decreased corneal sensitivity.[4] Furthermore, a decrease in intraocular pressure occurs during the third trimester of pregnancy.[1,2,5] Preexisting ocular diseases (e.g., diabetic retinopathy and uveitis) can be exacerbated during pregnancy. In addition to these pregnancy-induced ocular changes, preeclampsia and its complications can be associated with a variety of visual changes.[6-10] Because the obstetrician/gynecologist may occasionally be confronted with a preeclamptic woman who suffers visual disturbances, this review aims to summarize existing information described in the literature. The search strategy included a MEDLINE search through December 2011, limiting to articles published in English language and reports including humans. The following Medical Subject Headings terms were used: "pregnancy induced hypertension," "(pre)eclampsia," and "visual disorders." Abstracts were reviewed for suitability. The references of the available articles were screened as well and were included when they represented a unique case report. This literature consisted mainly of isolated case reports or small case series. Not all single case reports we encountered were included in this review because often case reports described nearly identical features, and we decided to include reports that gave the most information on a particular case/condition. For each condition, we described its epidemiology, pathogenesis, and clinical manifestations.

(Pre)eclampsia

Some form of hypertension complicates 5% to 7% of all pregnancies and is one of the leading causes of maternal and fetal morbidity and mortality worldwide.[11,12] Preeclampsia is a pregnancy-specific disorder, characterized by hypertension and proteinuria after midgestation. The exact pathophysiology of preeclampsia remains to be elucidated but is considered to entail reduced organ perfusion and endothelial dysfunction.[13,14] Several maternal organs can be affected, including the brain in the form of eclampsia, which is marked by tonic-clonic convulsions. Accompanying symptoms include visual abnormalities, severe headache, nausea, vomiting, and altered mental state. Eclampsia occurs in approximately 0.5% of women with mild preeclampsia and in approximately 2% to 3% of those with severe preeclampsia.[12] The HELLP syndrome is characterized by Hemolysis, Elevated Liver enzymes, and Low Platelet count, and this syndrome significantly increases the risk of preterm birth and perinatal mortality rate compared with isolated preeclampsia. Delivery remains the only cure for preeclampsia/HELLP syndrome with careful scrutiny regarding blood pressure control and seizure prophylaxis, as well as monitoring of fetal well-being.[13] Neurologic or ophthalmic entities that have been associated with (pre)eclampsia include cortical blindness, serous retinal detachment, Purtscher-like retinopathy, central retinal vein occlusions, and retinal or vitreous hemorrhages.[8,9,15] Ensuing visual symptoms have been described to occur in approximately 25% of preeclamptic[13] and 19% to 45% of eclamptic women,[15,16] and include blurry vision, diplopia, amaurosis fugax, photopsia, and scotomata, including homonymous hemianopsia. The neurological and ophthalmic entities associated with (pre)eclampsia can arise from different parts of the visual pathway and are described in more detail in the following sections.

The visual pathway

The visual pathways emerge from the eye as the optic nerve, pass the optic chiasm adjacent to the pituitary gland, continue as the optic tract, move through the lateral geniculate nucleus of the thalamus, optic radiations, and end in the primary visual cortex (also known as V1 or striate cortex) (Fig. 1). The primary visual cortex is situated in the most posterior part of the occipital lobes and is the first cortical area to receive visual information. The primary visual cortex subsequently projects visual information via the secondary visual cortex (V2) to higher-order visual areas in the occipital, parietal, and temporal lobes (Fig. 2). These higher-order visual areas are each involved in the processing of specific aspects of visual information. Postchiasmal lesions of the visual pathway can cause homonymous visual field defects.[17,18]

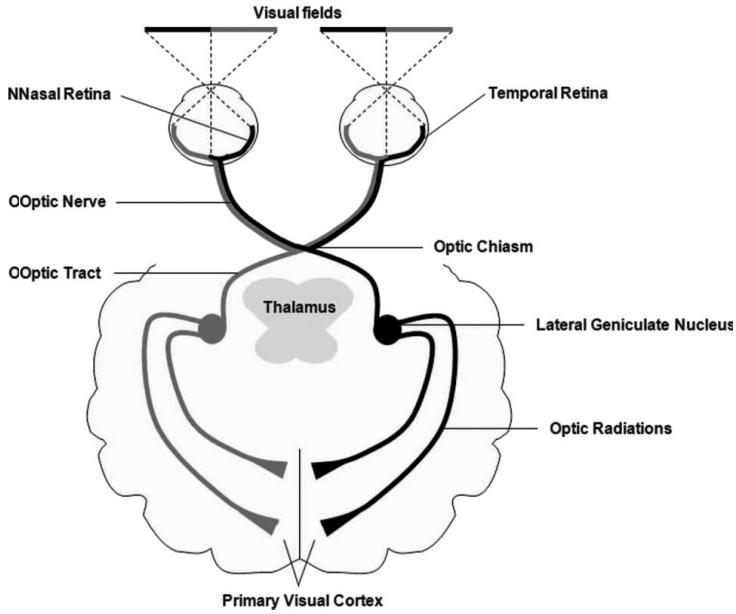

Figure 1. Visual pathways from the eyes to the primary visual cortex.

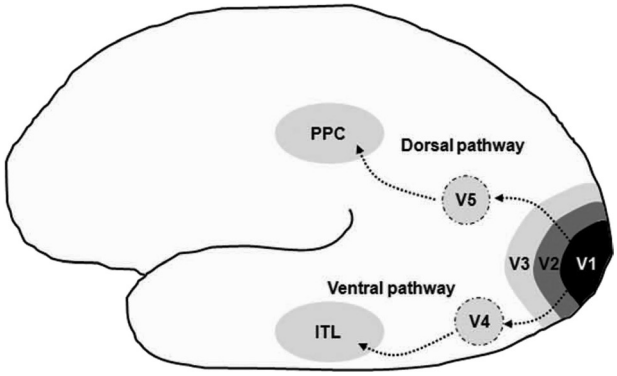

Figure 2. Visual information streams from the primary visual cortex to the higher-order visual areas. The primary visual cortex (V1) projects visual information via the secondary visual cortex (V2) and the association visual cortex (V3) to higher-order visual areas in the occipital, parietal and temporal lobes using a dorsal and a ventral stream. The ventral stream passes V4 (in the ventral occipito-temporal region) and ends up in high-order visual areas in the inferior temporal lobe (ITL). The dorsal stream passes V5 (in middle temporal area) and ends up in higher-order visual areas in the posterior parietal cortex (PPC).

Cortical blindness

Epidemiology

Cortical blindness is one of the reported complications of (pre)eclampsia. Cunningham et al reported that 15% of eclamptic women were affected.[19] The incidence of cortical blindness in preeclampsia without seizures is likely substantially lower. This type of blindness is caused by dysfunction of the optic radiations, primary and secondary visual cortexes, and high-order visual areas of the parieto-occipital lobes, hence its name.[20,21] Cortical blindness is per definition associated with an intact pathway from the eye to the lateral geniculate bodies, and therefore, the pupillary light responses and ocular motility remain intact.[21,22] Normal ophthalmoscopic findings exclude an ophthalmic cause of blindness.[19,23,24]

Clinical manifestation

Cortical blindness has been described to occur not only several hours before or after eclamptic seizures but also, although rarely, for several days up to weeks postpartum.[24-26] The bilateral vision loss often begins with blurry vision and progresses within a couple of hours to bare light perception. Prodromal symptoms are similar to imminent eclamptic seizures and include nausea, vomiting, and severe (often frontal) headache. Blindness can also be the presenting symptom in preeclampsia.[27] Cortical blindness is an anxiety-provoking condition that fortunately resolves completely in most cases. Sometimes, and as presented in the case report, the patient is unaware of her blindness and feels that she can see (visual anosognosia or Anton syndrome), indicating involvement of the visual association cortex (Fig. 2).[26,28]

A computerized tomographic (CT) scan may show low-density areas (corresponding with cerebral edema) in both occipital lobes, which can extend to the frontoparietal and parietal lobes. On T2-weighted MRI, these areas appear hyperintense and are mainly located in the parieto-occipital lobes, but they can also occur in the frontal and temporal lobes and basal ganglia.[24,29-31] Cases have been described in which the CT scan appeared normal, whereas major abnormalities were found on T2-weighted MRI,[23,29,32] emphasizing the superiority of MRI for the evaluation of sudden bilateral vision loss in (pre)eclampsia. Delefosse et al reported an interesting case of a woman who had had preeclampsia, and whose hypertension recurred 3 weeks postpartum.[24] This case illustrated an uncommon evolution of severe preeclampsia with secondary onset of neurologic symptoms, including cortical blindness, which the authors determined to be associated with intrauterine retention of placental products. After curettage and removal of the placental fragments, the woman was able to distinguish bright light within 12 hours. Visual acuity returned to normal after 4 days.

Pathophysiology

Cortical blindness in (pre)eclampsia is usually related to the occurrence of the Posterior Reversible Encephalopathy Syndrome (PRES).[33,34] PRES is a clinical-neuroradiological entity characterized by headache, vomiting, seizures, altered mental status, and visual abnormalities (including blurry vision, homonymous hemianopsia, visual neglect, cortical blindness, and visual anosognosia), together with cranial imaging findings consistent with vasogenic edema.[33,34] PRES is thought to be due to loss of cerebral autoregulation in the context of endothelial dysfunction.[35-38] Increased blood pressure overcomes the cerebrovascular autoregulation, resulting in hyperperfusion and vasogenic edema.[36,37,39] This edema is presumably the cause of the neurologic symptoms of PRES, including cortical blindness, when the edema affects the primary visual cortex in the occipital lobes.

The occipital lobes seem more susceptible to autoregulation breakthrough and subsequent hyperperfusion than other regions.[19,28,29,40] This may be explained by differences in innervation: the internal carotid system is better supplied with sympathetic nerves than the vertebrobasilar system.[40] With acute hypertension, protective sympathetic nerves enhance vascular autoregulation.[8] Severe autoregulation breakthrough can cause fibrinoid necrosis of the vessel wall and subsequent extravasation leading to accumulation of extracellular fluid, hypoperfusion of affected areas by increased hydrostatic pressure, and petechial hemorrhages.[19,28,41]

Prognosis

Lowering blood pressure and preventing seizures with magnesium sulfate are the primary objectives in (pre)eclamptic patients with cortical blindness. Women typically regain normal vision within a couple of hours or days after the onset of treatment. Complete resolution of the high-intensity signals (cerebral edema) on MRI can usually be expected as well.[19,42] However, residual symptomatic visual field defects and visuospatial deficits have been described in patients with concomitant presence of multiple bilateral parieto-occipital hemorrhages.[25,43] Although most women can expect full recovery, the combination of (pre)eclampsia-related cortical blindness and involvement of the eye (often a retinal detachment and/or Purtscher-like retinopathy) has been described to result in permanent visual impairment, and even blindness, in a few case reports.[19,44-46] In such cases, the bilateral retinal detachments and Purtscher-like retinopathies were accompanied by brain infarcts.[44,45] Murphy and Ayazifar reported diffuse optic disc and intraretinal and cerebral hemorrhages in a woman with eclampsia and HELLP syndrome who subsequently suffered permanent visual deficits.[46]

Balint's syndrome

Another complicated form of cortical blindness is Balint's syndrome, a rare condition

usually associated with cerebral infarction or neurodegenerative diseases. This triad is characterized by simultanagnosia (inability to integrate complex visual scenes), ocular apraxia (inability to voluntarily control gaze), and optic ataxia (inability to benefit from visual guidance in reaching an object). Balint's syndrome is believed to be the result of a bilateral dysfunction of the dorsal stream in the parieto-occipital cortices (Fig. 2).[47] To date, only 2 reports have described the development of this syndrome in an eclamptic woman.[48,49] The second case report was written in Italian, but it has an English abstract. Because of the rarity of Balint's syndrome in eclampsia, this case report is included in this review. In both reports, bilateral parieto-occipital infarcts were seen on CT scanning, which might reflect infarction of the cerebral posterior border zone region. Both women experienced improvement in object recognition and other higher-order visual functions after several months. One of the few existing studies evaluating neurologic functioning in eclampsia examined 30 women for the presence of simultanagnosia.[50] This evaluation took place in the first hours after eclamptic convulsion(s), after magnesium sulfate administration but before MRI scanning. All but one appeared to experience simultanagnosia. However, none of these women had ocular apraxia or optic ataxia to complete Balint's syndrome. Unfortunately, whether these women were symptomatic was not indicated in this article. In 87% of the women, diffusion weighted imaging showed reversible bilateral focal hyperintensity lesions, which correlated well with the simultanagnosia. On repeat examination (after 3–5 days), simultanagnosia had resolved in all cases.

Serous retinal detachment

Epidemiology

Serous retinal detachment is a well-documented ocular manifestation occasionally seen with (pre)eclampsia. The occurrence in women with preeclampsia varies from <1% to 3%. In eclamptic patients, this rate is 5 to 10 times higher.[51-55] Routine ophthalmic examination in 71 women admitted with severe preeclampsia/eclampsia revealed a prevalence of 32%.[56] Of the 3 cases presented in more detail by the authors, only one woman reported visual symptoms. Unfortunately, whether the 71 studied women experienced visual symptoms was not mentioned in the article.

Clinical manifestation

Preeclampsia-related serous retinal detachment has been described to occur before, during, or after delivery.[57-62] In an interesting case report, bilateral serous retinal detachment was reported to reveal an occult pregnancy.[63] The onset of preeclampsia-related serous retinal detachment is sudden, the main symptoms are a visual field defect and loss of visual acuity. Photopsia and floaters, which are usually present in rhegmatogenous retinal detachment, are absent.[9,52,53,61,64-66] Retinal detachments in preeclampsia are often bilateral,

but unilateral detachment can also occur. Occasionally, retinal detachments coexist with cortical edema. Fundoscopy reveals, in addition to the detached retina, retinal and/or macular edema, exudates, hemorrhages, and cotton wool spots (retinal nerve fiber swelling due to ischemia).[9,52,53,58,64,67,68]

Pathophysiology

The retinal pigmented epithelium (RPE) facilitates the exchange of water, salts, nutrients, and metabolites between the retina and the choroid, and prevents the accumulation of fluid in the subretinal space, which is a potential space in healthy eyes. Tight junctions between the RPE cells form the blood-retinal barrier,[53] which can be disturbed by conditions such as severe acute hypertension, inflammation, infection, neoplasm, hypoproteinemic states, and subretinal neovascularization.[69] One or more of these processes is likely at play in preeclampsia.[70] Some reports suggest hormonal involvement (by releasing endogenous vasoconstrictors).[8,62] Vasospasm, generalized or localized, is seen in the choroidal arterioles and the central retinal and posterior ciliary arteries.[8,9,68] Intense spasm of the choroidal arterioles results in choroidal ischemia, and this increases vascular permeability.[53] Subsequently, serous fluid accumulates in the subretinal space, separating the retina from the RPE.[71] Photoreceptors located in the retina become devoid of nutrients and stop functioning.[53]

Fluorescein angiography in (pre)eclamptic women with serous retinal detachment demonstrates areas of choroidal hypo- and nonperfusion (Elschnig spots).[9,68,71] Abnormalities in preeclamptic women may also be found by multifocal electroretinography and optical coherence tomography.[66,72,73] Changes in ocular blood flow, indicating vasospasm, can be found with color flow Doppler ultrasonography.[9,74] As an alternative to the vasospasm theory, it is suggested that hyperperfusion and breakthrough in autoregulation of orbital vessels, especially choroidal arterioles, increase permeability of retinal and choroidal arterioles, causing retinal edema and serous detachment. Systemic magnesium sulfate therapy has been described to significantly increase retinal perfusion. Simultaneously, headache and visual symptoms resolve.[74] This effect of magnesium sulfate could explain why systemic treatment of (pre)eclampsia is more effective in reducing visual symptoms than specific ocular treatment.

Prognosis

In general, the visual acuity gradually improves and the visual field defects disappear within 3 months postpartum, and the patients regain normal vision.[8,53,59,61,71,73] Within 1 week from the initial ophthalmoscopic examination, three-quarters of the serous retinal detachments will have resolved.[56]

Although visual acuity recovers and the retinal detachment spontaneously resolves

in most patients, a small percentage will show retinal abnormalities during follow-up weeks to 1-year postpartum with subtle changes in visual acuity. None of the reviewed articles mentioned accompanying visual symptoms in the following months; therefore, it is likely that the subtle visual changes have no consequences in daily life.

Purtscher-like retinopathy

Purtscher's retinopathy, first reported by Otmar Purtscher in 1910, is a specific appearance of the fundus characterized by multiple areas of retinal whitening and intraretinal hemorrhages, usually caused by trauma.[44,75] Systemic conditions, including acute pancreatitis, renal failure, fat embolism syndrome, connective tissue disorders, and (pre)eclampsia, have been associated with the same specific appearance of the fundus, and which is therefore named Purtscher-like retinopathy.[75] Only a few case reports of Purtscher-like retinopathy in (pre)eclamptic patients could be retrieved from the literature,[44,45,76-78] suggesting that it is an extremely rare complication of (pre)eclampsia.

Clinical manifestation

In the 6 (pre)eclamptic patients with Purtscher-like retinopathy described in the literature, visual abnormalities developed in the first 24 hours after urgent cesarean section.[44,45,76-78] Complaints included blurry vision, floaters, and complete bilateral vision loss. On ophthalmic examination, the visual acuity ranged from light perception to 20/400 and was bilateral in all cases.[44,45,76-78] In all patients, fundoscopic examination revealed multiple, discrete areas of retinal whitening, so-called Purtscher flecken, mostly within the macula and around the optic disc.[44,45,76-78] In addition, small, characteristic, flame-shaped hemorrhages were found in 4 of the patients.[44,45,76,77] Fluorescein angiography revealed capillary nonperfusion in areas of retinal whitening, and narrowing or occlusion of retinal arterioles.[44,76]

Pathophysiology

Agrawal and McKibbin postulate that, considering the specific appearance of the fundus, Purtscher flecken may be caused by embolic occlusion of the precapillary arterioles of the retina by fat, air, platelets, or leukocyte aggregates.[75] The latest hypothesis is the formation of leuko emboli by complement activation.[44,77] Previous reports suggest a role of amniotic fluid in activating complement and inducing leuko-embolization, which may contribute to complications of late pregnancy, such as Purtscher-like retinopathy.[44,45,77]

Prognosis

Improvement of visual acuity can be seen within a couple of weeks after the initial insult. Some recovery of visual acuity is accompanied by partial resolution of the retinal whitening and delayed filling of the retinal vasculature.[44,76,77] Apart from treating the

underlying systemic condition, no specific treatment is available, although it has been suggested that highdose steroids might be effective.[75] Unfortunately, none of the reported 6 (pre)eclampsia-related cases experienced complete recovery of visual acuity. In fact, one preeclamptic woman had unchanged visual acuity of 4/200 in both eyes 2 months postpartum.[44] Another preeclamptic woman, who also suffered pancreatitis, experienced slight improvement from bare light perception in the acute phase to counting fingers at 1 meter (i.e., a visual acuity of 1/60) in both eyes after 6 months.[77]

Central retinal vein occlusion

Two case reports describe sudden vision loss in preeclamptic women caused by central retinal vein occlusion (CRVO).[79,80] Both women developed bilateral loss of vision 10 to 21 days postpartum. At the onset of vision loss, blood pressure was normal in both cases. In both women, ophthalmologic examination revealed the classical picture of CRVO, that is, multiple retinal hemorrhages in all 4 quadrants, venous dilatation, and macular edema. In the next months, the hemorrhages resolved and macular edema reduced. Visual acuity improved significantly, but did not return to normal. The pathophysiology of CRVO in (pre)eclampsia is not fully understood. Although the name CRVO suggests otherwise, CRVO is primarily an arterial problem. Central retinal artery thickening is thought to cause compression of the central retinal vein thereby leading to venous occlusion. Interestingly, the risk factors for CRVO, hypertension, and coagulation disorders are also associated with preeclampsia.[81]

Retinal and vitreous hemorhages

Retinal and vitreous hemorrhages, preceding the presentation of preeclampsia, although rare, have been described each in the literature once. One case report described sudden vision loss in the left eye of a normotensive pregnant woman.[82] Ophthalmologic examination revealed white-centered retinal hemorrhages (Roth spots). Within 48 hours, this previously healthy pregnant woman developed preeclampsia. During the first 6 months after delivery, visual acuity returned to normal, and the retinal hemorrhages resolved. The other case report described sudden vision loss in the left eye of a normotensive pregnant woman, resulting from a vitreous hemorrhage.[83] This vitreous hemorrhage gradually resolved over the next 2 weeks. However, the woman developed preeclampsia with HELLP syndrome, another 2 weeks later, which may or may not have been coincidental.

Conclusions

Visual symptoms during preeclampsia are a frequent phenomenon. Therefore, the obstetrician/gynecologist can encounter women with serious, and sometimes debilitating, pathology of the visual pathways. This review describes the most common presentations,

including cortical blindness, serous retinal detachment, Purtscher-like retinopathy, central retinal vein occlusion, and retinal and vitreous hemorrhages. Unfortunately, the existing literature largely consists of single case reports or small case series. Routine follow-up with standardized history taking of visual complaints/ symptoms and standardized ophthalmic examination, was not necessarily performed or described. Moreover, the frequency of occurrence of the reported visual manifestations in uncomplicated pregnancy and in the healthy young population is largely unknown. All this hampers drawing firm conclusions regarding the etiology of visual disturbances in preeclampsia and the existence of a causal relationship.

In general, aside from lowering blood pressure and preventing (further) seizures with magnesium sulfate, no specific therapy seems indicated for (pre)eclamptic women who experience visual changes. When not rapidly transient, ophthalmic evaluation is helpful in distinguishing the underlying pathology. MRI may be helpful in delineating features of cerebral edema/ PRES in the occipito-parietal lobes. In most cases, visual acuity returns to normal within weeks to months after the onset of symptoms. Rarely, permanent visual impairment in (pre)eclamptic women has been described to occur, generally associated with occipital hemorrhages or brain infarcts, alone or in combination with retinal hemorrhages (e.g., Purtscher-like retinopathy).

The follow-up in the described case reports typically included ophthalmoscopic examination and, in some cases, an MRI scan. However, a few articles report visual symptoms experienced by the formerly (pre)eclamptic women. The fact that most articles do not mention any visual symptoms during follow-up may tentatively lead to the conclusion that most women with visual disturbances during (pre)eclampsia experience no permanent visual symptoms.

Health care providers such as emergency room physicians, obstetricians, family physicians, neurologists, and ophthalmologists should be aware that acute onset of visual symptoms in pregnant women can be the first sign of (pre)eclampsia. An ophthalmologist or neurologist faced with such a woman should consult an obstetrician or refer the woman without delay. Given that visual changes are a diagnostic criterion for severe preeclampsia, obstetricians should appreciate the significance of these changes and discuss appropriate diagnostic options with the ophthalmologist. Affected women can be reassured that most cases are transient. In addition, obstetricians should refer pregnant women with persistent visual symptoms to an ophthalmologist, as some ophthalmic entities may require specific therapy (e.g., central retinal vein occlusion) or follow-up by an ophthalmologist.

Continuation vignette

The patient was administered magnesium sulfate for seizure prevention, and her blood pressure was lowered with intravenous antihypertensive medication. Labor was induced, and she spontaneously delivered a healthy female infant of 2850 g. In the 3 days after

delivery, she gradually regained complete vision. MRI obtained 6 weeks postpartum revealed complete resolution of cerebral edema and no other abnormalities.

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7

LONG-TERM VISUAL FUNCTIONING AFTER ECLAMPSIA

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Abstract

Objective

Complete neurocognitive recovery after eclampsia has been questioned with the expression of neurocognitive deficits by affected women and demonstration of cerebral white matter lesions on magnetic resonance imaging years after eclampsia. We hypothesized that formerly eclamptic women may experience impaired vision-related quality of life (QOL) and visual field loss as a result of the presence of such lesions in the cerebral visual areas.

Methods

Using the National Eye Institute Visual Function Questionnaire-39/Nederlands questionnaire, vision-related QOL was compared between formerly eclamptic women and control participants after normotensive pregnancies. Furthermore, in formerly eclamptic women, visual fields were assessed using automated perimetry, and presence of white matter lesions was evaluated using cerebral magnetic resonance imaging. Presence of a relationship between these lesions and National Eye Institute Visual Function Questionnaire-39/Nederlands scores was estimated.

Results

Forty-seven formerly eclamptic women and 47 control participants participated 10.1 ± 5.2 and 11.5 ± 7.8 years after their index pregnancy, respectively. Composite scores and 4 out of 12 National Eye Institute Visual Function Questionnaire-39/Nederlands subscale scores were significantly lower in formerly eclamptic women than in control participants ($P<.01$ for composite scores). This could not be explained by visual field loss, because all formerly eclamptic women who underwent perimetry (n=43) demonstrated intact visual fields. White matter lesions were present in 35.7% of formerly eclamptic women who underwent magnetic resonance imaging (n=42) and were associated with lower vision-related QOL scores ($P<.05$ for composite scores).

Conclusion

Formerly eclamptic women express lower vision-related QOL than control participants, which seemed at least partly related to the presence of white matter lesions. However, such women do not have unconscious visual field loss. Vision-related QOL impairment expressed by formerly eclamptic women may therefore be related to problems with higher-order visual functions.

Introduction

Visual disturbances are relatively common during the acute phase of eclampsia, including transient cortical blindness, scotomata, visual neglect, and blurred vision, and can often be attributed to the presence of cerebral edema.[1] Although the exact pathophysiology of eclampsia remains to be elucidated, it is considered to be an expression of the posterior reversible encephalopathy syndrome.[2] As its name suggests, this syndrome is thought to be a completely reversible condition. However, the reversibility of this syndrome, and eclampsia in particular, has been questioned lately.[3] In previous studies, one-fourth of eclamptic women showed persistent cerebral white matter lesions and brain tissue loss on magnetic resonance imaging (MRI) 6 weeks postpartum.[4,5] Furthermore, several years after eclampsia, such lesions are more prevalent in formerly eclamptic women compared with women with normotensive pregnancies.[6]

In general, visual disturbances expressed by patients with posterior reversible encephalopathy syndrome or eclampsia are known to be transient. However, permanent visual field abnormalities, in particular hemianopia, but also permanent blindness, have been described and are thought to be related to persistent lesions in the visual cortex.[3,7] Moreover, in a Scandinavian follow-up study, 11% of formerly eclamptic women reported persistent visual disturbances.[8] However, median follow-up time of these studies was relatively short, being less than 1 year.

When located in the visual pathway from the optic nerve to optic radiation, white matter lesions may cause (un)conscious visual field defects or impairment of higher-order visual functions such as visual perception and spatial orientation. Visual disturbances might be a contributing factor related to persistent complaints described by some formerly eclamptic women such as poor concentration and limited attention span.[8,9] We hypothesized that years after eclampsia decreased vision-related quality of life (QOL) and visual field loss may be experienced as a result of the presence of white matter lesions in the cerebral visual areas. We choose to use a validated vision-related QOL questionnaire to evaluate these women's own perception of daily functioning in relation to vision. The questionnaire determines the influence of visual disability and visual symptoms on generic health domains such as emotional well-being and social functioning in addition to task-oriented domains related to daily visual functioning. Subsequently, the relationship of this self-perceived vision-related QOL with the prevalence of visual field defects and cerebral white matter lesions was assessed.

Material and methods

Women with a diagnosis of eclampsia in their medical history between 1988 and 2008 were identified in the University Medical Center Groningen, VU University Medical Center Amsterdam and Isala Clinics Zwolle. These hospitals are teaching hospitals that serve as tertiary perinatal referral hospitals in The Netherlands. Eclampsia was defined according

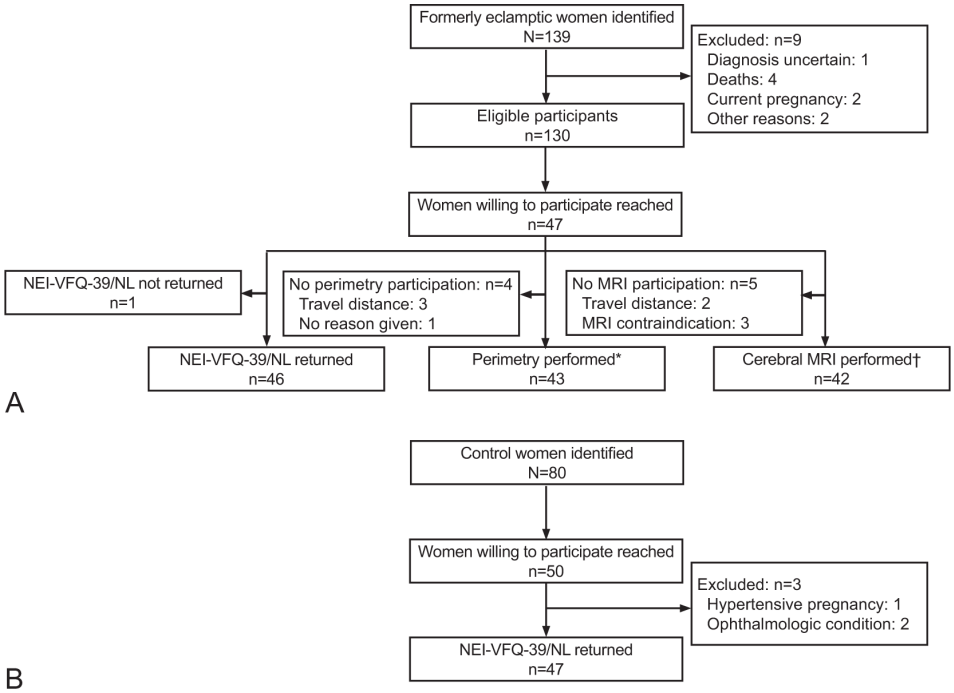

Figure 1. Flow diagram illustrating the inclusion and exclusion process of study participants. **A.** The selection of formerly eclamptic women. **B.** The selection of control participants. *Forty-two of these women returned the National Eye Institute Visual Function Questionnaire-39 (NEI-VFQ-39/NL). †Forty-one of these women returned the NEI-VFQ-39/NL.

to the definition of the International Society for the Study of Hypertension in Pregnancy.[10] Exclusion criteria included pre-existing glaucoma or other conditions known to be related to visual field defects, epilepsy or other neurological disorders including a known cerebrovascular accident, intracranial infections, or a history of any cranial neurosurgical procedure. Also, current pregnancy and an age of younger than 18 years were used as exclusion criteria.

In the three participating hospitals, electronic admission, diagnosis, and delivery databases are kept up to date, which were used to identify eligible study participants (Fig. 1). In total, 133 women were diagnosed with eclampsia in these three hospitals between 1988 and 2008. In addition, six women who delivered in other hospitals than the participating clinics and who had heard about this study requested to participate in the current study, which was allowed.

Medical records were reviewed for accuracy of diagnosis of eclampsia and to extract clinical and demographic characteristics. On review, one woman was excluded because a diagnosis of eclampsia could not be confirmed. Three women were described as having had generalized myoclonic twitches while remaining conscious. Because these twitches suggest cerebral involvement, these women were not excluded from the study although they had

not experienced tonic–clonic seizures. Four women had died in the interim, two of whom as a result of cerebral complications resulting from eclampsia, one resulting from breast cancer, and one attributable to cervical cancer years after her pregnancy. Four other women were excluded, two women as a result of a current pregnancy, one because of a cerebrovascular accident, and one because she did not speak Dutch.

This resulted in 130 formerly eclamptic women who were eligible to participate. These women received a written invitation for participation in this study. Of these women, 47 women could be reached and were willing to participate. Several women, who decided not to take part in the study, mentioned travel distance and time commitment as the main reason for nonparticipation. Some other women did not want to be confronted with their medical history.

In addition to these formerly eclamptic women, 47 parous control women who had normotensive pregnancies participated in this study (Fig. 1). These women were recruited among hospital personnel of the University Medical Center Groningen. In total, 80 such women were randomly selected and invited to participate, of which 50 decided to take part in the study. One of them was excluded as a result of a history of hypertension in pregnancy. An additional two had to be excluded as a result of ophthalmologic conditions known to be associated with visual field impairment.

Both formerly eclamptic and control participants filled out a questionnaire related to their obstetric and current and past medical history. Approval for this project was obtained from the Medical Ethics Committee of the University Medical Center Groningen, and informed consent was obtained from the participants.

Vision-specific health-related QOL was compared between formerly eclamptic and parous control participants using the validated Dutch translation of the National Eye Institute Visual Function Questionnaire-39/Nederlands.[11] The English version of this questionnaire was developed at RAND Health under the sponsorship of the National Eye Institute and was designed to measure the dimensions of self-reported vision targeted health status.

The National Eye Institute Visual Function Questionnaire-39/Nederlands consists of a base set of 25 questions and 14 additional questions generating the following vision-targeted subscales: general health, general vision, near and distance activities, driving difficulties, peripheral vision, color vision, ocular pain, vision-related role function, vision-related dependency, vision-related social functioning, and vision-related mental health. Each question was scored on a 0 to 100 scale with 0 being the worst and 100 being the best possible score. Each subscale score was calculated by averaging the scores of the questions that constitute that particular subscale. The composite score was calculated by averaging all vision-targeted subscale scores, excluding the general health subscore.

The National Eye Institute Visual Function Questionnaire-39/Nederlands was filled out by 46 formerly eclamptic women, of which 42 had participated in visual field testing. One of the women, who had undergone perimetry and MRI, did not return the questionnaire.

Three women filled out the questionnaire but did not participate in visual field testing as a result of travel distances. Results were compared with scores from 47 parous control women, who participated in vision-related QOL assessment only.

The prevalence of visual field defects was assessed in 43 formerly eclamptic women at the Department of Ophthalmology of the University Medical Center Groningen using automated perimetry. The control group did not undergo visual field testing as a result of an expected very low prevalence of visual field defects in these participants. This presumption was based on data from the Rotterdam Study, which showed a prevalence of visual field loss of 3% in the general elderly population (55–64 years).[12] In our group of healthy women of on average 40 years old, the prevalence of visual field defects would likely be less than 3% and probably even closer to 1%. Based on literature indicating approximately a 50% prevalence of visual field defects in patients with former stroke,[13] we anticipated that at least 25% of formerly eclamptic women who demonstrate cerebral white matter lesions would experience some degree of visual field loss. Although a formal power calculation could not be performed as a result of lack of available data in the literature, we considered the number of 43 formerly eclamptic women to evaluate whether the prevalence of visual field loss in such women is clinically relevant.

An assistant of the Department of Obstetrics and Gynecology was trained by a certified technician in conducting automated perimetry using the Humphrey Field Analyzer. The visual field was tested with a 52-point suprathreshold test that covered the central visual field with a radius of 24°, a test identical to the test used in the Rotterdam Study.[14] Visual field loss was defined as nonresponse to a light stimulus of 6 dB above a threshold-related estimate of the hill of vision in at least three contiguous test points or four including the blind spot.

Visual field testing was performed on one eye at a time with the contralateral eye covered. The patient was asked to look straight ahead while lights were projected at different places in the perimeter. Participants were instructed to click a button every time they noticed a projected light.

Test reliability was evaluated using the reliability indices for false-positive and false-negative answers; fixation stability was observed by the perimetrist. In case of visual field loss or an unreliable test result (more than 33% false-positive or false-negative catch trials or poor fixation as observed by the perimetrist), the test was repeated in the same session after a break. Visual field loss on an initial test is, as a result of a learning effect, quite common and should always be confirmed or falsified.[15]

Cerebral MRI was performed in 42 of the 47 formerly eclamptic participants as part of an ongoing follow-up study assessing white matter lesions after eclampsia.[6] These MRI scans were made on a 3-Tesla MRI system using the following sequences: T1, T2, proton density, and fluid-attenuated inversion recovery. The presence of white matter lesions was rated by an experienced neuroradiologist as previously described.[6,16] Briefly, such lesions were considered present if hyperintense on T2- and proton densityweighted images and

not hypointense on T1-weighted images. The presence of subcortical white matter lesions was evaluated for the following locations: frontal, parietal, temporal, occipital and insular lobe, brain stem, and cerebellum. To correct for inclusion of partial volume, women with two small white matter lesions or less were considered as negative. Descriptive statistics such as demographic information and scores on the National Eye Institute Visual Function Questionnaire-39/Nederlands were presented as means±standard deviations for continuous variables and percentages for dichotomous variables.

Demographic data and National Eye Institute Visual Function Questionnaire-39/Nederlands subscale and composite scores were compared between formerly eclamptic and control women using Fisher's exact test, independent Student's *t* test, or Mann-Whitney *U* tests, where appropriate. The relation between National Eye Institute Visual Function Questionnaire-39/Nederlands scores and presence of white matter lesions was assessed using Mann-Whitney *U* tests. Differences were considered statistically significant at $P \leq .05$. Data analyses were performed using SPSS for Windows 18.

Results

In this study, 47 formerly eclamptic women participated. Of these women, 46 completed the National Eye Institute Visual Function Questionnaire-39/Nederlands, 43 underwent perimetry, and 42 underwent cerebral MRI. In total, 47 parous control women who did not experience a hypertensive pregnancy filled out the National Eye Institute Visual Function Questionnaire-39/Nederlands.

Table 1 shows baseline characteristics of the study participants. Mean age and elapsed time since index pregnancy at the time of participation were similar for both groups. Also, the percentage of white women and nulliparity at the time of index pregnancy were comparable between the groups. However, as may be expected, birth weight and estimated gestational age at delivery were significantly lower in formerly eclamptic women compared with parous control participants.

Vision-related QOL may be influenced by visual field defects and impairment of higher-order visual functions and therefore was assessed in formerly eclamptic women

Table 1
Characteristics of formerly eclamptic and parous control participants

	Eclampsia (n=47)	Control (n=47)	P value
Age (y)	40.3 ± 7.0	41.7 ± 8.1	.362
White	91.5 (83.5–99.5)	100.0 (100.0–100.0)	.117
Elapsed time since index pregnancy (y)	10.1 ± 5.2	11.5 ± 7.8	.631
Nulliparous	87.2 (77.7–96.8)	80.9 (69.7–92.1)	.574
Estimated gestational age at delivery (wk)	33.9 ± 4.7	39.8 ± 1.9	<.001
Birth weight of child (g)	2,022 ± 1,054	3,522 ± 544	<.001

Data are mean±standard deviation or % (95% confidence interval) unless otherwise specified.

and parous control participants. Compared with parous control participants, formerly eclamptic women had significantly lower composite scores as well as lower scores on all subscales. This difference was significant for the composite of the National Eye Institute Visual Function Questionnaire-39/Nederlands and for four of 12 subscales, specifically, general health, vision-related social functioning, driving, and peripheral vision (Table 2). For the subscales general vision, ocular pain, near and distance activities, vision-related mental health, vision-related role function, vision-related dependency, and color vision, the differences did not reach significance (Table 2).

Automated perimetry was performed in 43 formerly eclamptic women. At the first examination, nine of these women showed abnormal test results in at least one eye and one had an unreliable test result. On the repeat test, all had a reliable test result and none showed visual field defects.

In total, 42 formerly eclamptic participants underwent MRI, of which 15 (35.7%, 95% confidence interval [CI] 21.2–50.2%) demonstrated white matter lesions. All but two of these women (86.7%, 95% CI 69.5–100.0%) had these lesions in the frontal lobe and some in the parietal lobe (n=4; 26.6%, 95% CI 4.2– 49.0%), insular lobe (n=3; 20.0%, 95% CI 0.0–40.2%), or cerebellum (n=1; 6.7%, 95% CI 0.0–19.4%). No lesions were observed in the occipital, temporal lobe, or brain stem. Five formerly eclamptic women demonstrated white matter lesions in multiple brain regions (33.3%, 95% CI 9.5–57.2%).

Magnetic resonance imaging scans were available for 41 of the formerly eclamptic participants who also filled out the National Eye Institute Visual Function Questionnaire-39/ Nederlands. For three women, no scans were available as a result of general contraindications

Table 2
National Eye Institute Visual Function Questionnaire-39 subscale and composite scores of formerly eclamptic and parous control participants

	Eclampsia (n=46)	Controls (n=47)	P
General Health	67.3 ± 15.8	74.7 ± 14.0	.027
General Vision	80.5 ± 11.2	83.3 ± 9.3	.175
Ocular Pain	86.1 ± 17.1	90.2 ± 12.8	.380
Near Activities	93.2 ± 9.4	94.9 ± 6.1	.508
Distance Activities	92.9 ± 10.4	96.5 ± 4.1	.362
Social Functioning	98.3 ± 5.1	100.0 ± 0.0	.006
Mental Health	90.2 ± 12.1	93.5 ± 7.5	.181
Role Function	92.4 ± 12.8	95.9 ± 7.1	.327
Dependency	98.9 ± 3.0	99.5 ± 2.9	.142
Driving	75.6 ± 14.8	85.9 ± 11.1	.001
Color Vision	98.4 ± 8.2	100.0 ± 0.0	.151
Peripheral Vision	90.8 ± 17.0	98.4 ± 8.1	.002
Composite Score	90.9 ± 7.8	94.5 ± 3.5	.005

Data are mean±standard deviation unless otherwise specified.

for MR scanning and another two participants waived participation in MRI scanning as a result of travel distance. Fourteen (34.1%, 95% CI 19.6–48.6%) formerly eclamptic women who participated in the National Eye Institute Visual Function Questionnaire-39/Nederlands demonstrated white matter lesions. These women had lower subscale scores compared with formerly eclamptic women without lesions, except for the subscale color vision (Table 3). This difference was significant for the subscales general vision, near activities, vision-related role function, and peripheral vision. In addition, formerly eclamptic women with white matter lesions had a significantly lower composite score compared with those without lesions.

Discussion

Women who have experienced eclampsia report lower vision-related QOL on average 10 years after the index pregnancy compared with parous control participants who had normotensive pregnancies. In formerly eclamptic women, lower vision-related QOL was associated with the presence of cerebral white matter lesions. Because visual fields of these women were intact when examined by perimetry, this suggests that lower vision-related QOL was not the result of (un)conscious visual field loss related to white matter lesions.

Visual disturbances are relatively common during the acute phase of eclampsia, attributed to the presence of reversible cerebral vasogenic edema, mainly in the

Table 3
National Eye Institute Visual Function Questionnaire-39 subscale and composite scores of formerly eclamptic women with and without white matter lesions

	Eclampsia With White Matter Lesions (n=14)	Eclampsia Without White Matter Lesions (n=27)	P
General Health	62.5 (32.5-100.0)	65.0 (32.5-100.0)	.173
General Vision	75.0 (60.0-85.0)	85.0 (60-100.0)	.010
Ocular Pain	87.5 (37.5-100.0)	87.5 (50.0-100.0)	.530
Near Activities	91.7 (70.8-100.0)	95.8 (50.0-100.0)	.037
Distance Activities	91.7 (66.7-100.0)	100.0 (58.3-100.0)	.115
Social Functioning	100.0 (91.7-100.0)	100.0 (70.0-100.0)	.192
Mental Health	90.0 (70.0-100.0)	95.0. (45.0-100.0)	.175
Role Function	90.6 (56.3-100.0)	100.0 (50.0-100.0)	.028
Dependency	100.0 (93.8-100.0)	100.0 (87.5-100.0)	.460
Driving	66.7 (58.3-91.7)	75.0 (50.0-100.0)	.377
Color Vision	100.0 (75.0-100.0)	100.0 (50.0-100.0)	.659
Peripheral Vision	87.5 (50.0-100.0)	100.0 (25.0-100.0)	.045
Composite Score	86.3 (72.7-97.5)	95.3 (62.0-100.0)	.035

Data are median (range) unless otherwise specified.

(sub)cortical parieto-occipital and temporal regions.[17,18] The fact that a substantial percentage of formerly eclamptic women expresses persistent neurocognitive deficits and demonstrates white matter lesions[6] stimulated us to examine the possibility that these lesions could be associated with (un)conscious visual field loss.

Unawareness of visual field loss is a commonly described phenomenon[19,20] and can be attributed to occurrence of perceptual filling-in, in which missing information of a visual field defect is inferred from the surrounding intact visual field.[21] In addition, several animal studies have shown a marked potential for brain plasticity in response to both retinal and cortical lesions.[22,23] Interestingly, also human studies show evidence for (partly) functional recovery of visual field loss after cerebral scotomata.[24-26] Therefore, although formerly eclamptic women in our study did not demonstrate visual field loss on perimetry, one could hypothesize that small visual field defects might have been present in the period directly after the acute phase of eclampsia. We assessed visual fields years after eclampsia, a timeframe that might have allowed for rearrangement of visual pathways affected by white matter lesions. Hence, especially small visual field defects may have shrunken to undetectable abnormalities.[27]

Absence of identifiable lesions in the visual pathway from the optic nerve to optic radiation of our participants can obviously also explain a true absence of visual field defects after eclampsia. It seems plausible that white matter lesions after eclampsia are mainly located in the parieto-occipital region because vasogenic edema during the acute phase of eclampsia is most commonly observed here.[2] This edema, when severe enough, has been suggested to decrease regional cerebral perfusion pressure and blood flow to ischemic levels leading to areas of cytotoxic edema and infarction.[28] However, this study demonstrated only a few women with parietal white matter lesions and absence of such lesions in both the occipital and temporal lobe. Interestingly, white matter lesions were located in the frontal lobe in the majority of women with lesions. Because the primary visual cortex is located within the occipital lobe, these results can well explain the absence of visual field loss in formerly eclamptic women. Furthermore, the location of white matter lesions suggests a different or additional etiology of these lesions other than ischemia resulting from vasogenic edema. As a result of the relatively small group of lesion-positive women, further research addressing lesion location and its etiology after eclampsia is required.

Vision-related QOL was assessed using the National Eye Institute Visual Function Questionnaire-39/Nederlands, a commonly used questionnaire in the field of ophthalmology. A broad spectrum of ocular conditions has been shown to potentially alter vision-related QOL as measured by the National Eye Institute Visual Function Questionnaire-39/Nederlands, including glaucomatous and poststroke visual field loss,[29,30] congenital cataract,[31] and diabetic retinopathy.[32] In contrast with the objective visual field assessment as a measure of visual function, the National Eye Institute Visual Function Questionnaire-39/Nederlands

comprises aspects of daily functioning in relation to vision strictly from a participant's perspective. In addition to lower National Eye Institute Visual Function Questionnaire-39/ Nederlands composite scores in formerly eclamptic women, subscale scores related to general health, driving, peripheral vision, and vision-related social functioning were lower as well. This lower vision-related QOL appeared to be at least partly related to the presence of white matter lesions in formerly eclamptic women because women who demonstrated such lesions had a lower composite score as well as lower scores on one-third of the subscales compared with those without lesions. It should be noted that additional smaller, but still relevant, differences in subscale scores between the groups may have been missed as a result of insufficient power. However, although the number of women evaluated in this project may appear limited, in the context of the rare incidence of eclampsia, this study is considered sizeable.

The underlying mechanism by which white matter lesions might lead to lower vision-related QOL is still speculative at this moment because visual fields were intact. A possible explanation is that these lesions may interfere with neurocognitive functioning pertaining to higher-order visual functions, which might subsequently result in lower vision-related QOL and commonly reported complaints of formerly eclamptic women such as difficulties with reading texts. The majority of lesions in formerly eclamptic women appeared to be located in the frontal and parietal lobe, which are involved in higher-order visual functions, including visual memory and visuospatial processing.[33-35] Future research is needed to objectively assess such higher-order functions in formerly eclamptic and control women using a detailed neurocognitive test battery.

A few limitations of the current study should be noted. First, as a result of the retrospective nature of this study, no data are available on visual functioning before the index pregnancy. However, a prospective study design seems unfeasible in view of the rare incidence of eclampsia. Second, management and outcome of eclamptic women may have changed in the period during which our participants had eclampsia. However, this long period was required to obtain a sizeable study population, especially because the participation rate of eclamptic women was relatively low.

In conclusion, formerly eclamptic women have lower vision-related QOL, which seemed associated with the presence of cerebral white matter lesions. Because these lesions did not appear to induce visual field loss, further research is needed to unravel the underlying mechanism of lower vision-related QOL and its relationship with white matter lesions. However, our results are in line with the current doubts about the complete reversibility of eclampsia. In addition to earlier proposed long-term sequelae pertaining to self-perceived neurocognitive functioning, eclampsia may also affect visual functioning years after the complicated pregnancy.

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8

REGIONAL DISTRIBUTION OF CEREBRAL WHITE MATTER LESIONS YEARS FOLLOWING (PRE)ECLAMPSIA

Submitted

Abstract

Objective

Women with a history of (pre)eclampsia demonstrate an increased prevalence of cerebral white matter lesions (WML) compared to women who had normotensive pregnancies years following the index pregnancy. The pathophysiology of these WML is unclear but may be related to the predisposition for cerebro-/cardiovascular disease in such women and/or the occurrence of the posterior reversible encephalopathy syndrome (PRES) while pregnant. Assessing the distribution of WML in these women may give insight into both the pathophysiology as well as the possible consequences of these lesions and is therefore subject of this study.

Methods

Presence, severity and location of WML were determined in cerebral MRI scans of 64 formerly eclamptic, 74 formerly preeclamptic and 75 parous control women.

Results

Compared to parous controls, formerly (pre)eclamptic women demonstrated WML more often (35.0% versus 21.3%; $P<0.05$) and more severe (0.07 versus 0.02 mL; $P<0.05$). In all groups, the majority of WML was located in the frontal lobes, followed by the parietal, insular and temporal lobes.

Conclusion

Regional WML distribution was similar for all three groups and does not correspond to the typical occipito-parietal edema distribution observed in PRES. The frontal lobe distribution of WML in formerly (pre)eclamptic women may be a reflection of their predisposition for cerebro-/cardiovascular disease. Whether a history of PRES plays an additional role in this relation still remains speculative but seems less likely. The clinical implications of the presence and distribution of WML in our relatively young cohort remain so far unknown and future research is needed to examine the progress and possible sequelae of such lesions.

Introduction

The prevalence of cerebral white matter lesions (WML) is increased in formerly (pre)eclamptic women compared to women following normotensive pregnancies.[1, 2] Although the pathogenesis of these lesions remains to be elucidated, the increased propensity for cerebro-/cardiovascular disease in women with a history of preeclampsia[3] may be an associated factor in the development of WML.

In the elderly population WML are associated with cognitive decline and dementia[4] and such lesions are mainly located in the frontal lobe, followed by the parietal, temporal and occipital lobe.[5, 6] Although impaired subjective cognitive functioning in formerly eclamptic women has been previously reported,[7, 8] no significant differences were found for objective measures of sustained attention and executive functioning following eclampsia.[9] Therefore, the clinical implications of WML in formerly (pre)eclamptic women remain so far unclear and are subject of further investigation.

We hypothesize that an episode of posterior reversible encephalopathy syndrome (PRES), which is considered the underlying cause of the neurologic symptoms in eclamptic as well as some preeclamptic patients, may play an additional role in the development of WML.[10] In PRES, vasogenic edema has been suggested to potentially progress to such an extent that regional cerebral perfusion decreases and blood flow diminishes to ischemic levels leading to areas of cytotoxic edema and infarction and the development of WML in the long-term.[11, 12] This theory is supported by recent follow-up studies which have shown persistent neuroimaging abnormalities in a substantial part of former non-obstetric as well as obstetric PRES patients.[1, 11, 13-16] In addition to WML, various other imaging abnormalities have been observed in former PRES patients including infarction, atrophy, hemorrhage, and laminar necrosis. Moreover, permanent clinical symptoms have been found in some of these patients, including impaired self-perceived as well as objective neurocognitive functioning and visual disturbances.[7, 8, 17-19] Part of these clinical sequelae is thought to be related to permanent cerebral lesions. The exact relationship between neuroimaging findings and clinical symptoms following an episode of PRES remains, however, largely unknown.

Assessing the distribution of WML in formerly eclamptic and preeclamptic women may give insight into both the pathophysiology as well as the possible consequences of these lesions. Therefore, this study aimed to assess the distribution of WML in women who suffered eclampsia or preeclampsia compared to women who had normotensive pregnancies several years prior.

Materials and methods

Study participants

Participants were enrolled in this study as part of ongoing follow up studies assessing cerebral long-term consequences of (pre)eclampsia such as cerebral WML and neurocognitive

functioning.[1,2] Three groups of women were recruited for these studies, i.e. formerly eclamptic, formerly preeclamptic and parous control women.

Women with a diagnosis of eclampsia in their medical history between 1988 and 2005 were identified from the electronic admission, diagnosis, and delivery databases of the University Medical Center Groningen (UMCG). Recruitment and selection criteria have been published previously.[1, 2] In the meantime, additional formerly eclamptic women have been recruited to participate in our follow-up studies through collaboration with two other tertiary referral centers; the VU University Medical Center Amsterdam (VUmc) and Isala Clinics Zwolle. In addition, six women who delivered in other hospitals and who had heard about this study requested to participate in the current study, which was allowed. Recruitment and selection criteria of participating women with a diagnosis of preeclampsia or normotensive pregnancies in their medical history were reported previously.[2]

Briefly, medical records of formerly eclamptic and preeclamptic women were reviewed for accurateness of diagnosis and to extract clinical and demographic characteristics. Both eclampsia and preeclampsia were defined according to the definition of the International Society for the Study of Hypertension in Pregnancy (ISSHP).[20] For parous controls, records were evaluated to confirm that the pregnancy was indeed normotensive. Exclusion criteria included contra-indication for MR scanning, pre-existent epilepsy, a known cerebrovascular accident, demyelinating disorders, intracranial infections, a history of any cranial neurosurgical procedure, or the inability to understand Dutch. Only women who did not meet any of the exclusion criteria were invited to participate in the current study by mail.

Following the MRI procedure and after a period of rest blood pressure was measured manually using a sphygmomanometer. Subjects with a systolic blood pressure of ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg and/or currently using antihypertensive medication were diagnosed with current hypertension.[21]

Approval for this project was obtained from the Medical Ethics Committee of the UMCG and all participants signed informed consent.

Cerebral MR imaging protocol

Detailed MRI study protocols have been previously described.[1] Briefly, participants underwent MRI on a 3 Tesla MRI system (Philips Intera, Best, the Netherlands) using the following sequences: T1, proton density, T2, and fluid attenuated inversion recovery (FLAIR). Slice thickness was 5 mm with a 20% interslice gap. The presence, size and number of WML were rated by an experienced neuroradiologist, who was blinded for patient category as previously described.[1, 22] WML were considered present if hyperintense on FLAIR, T2- and proton density-weighted images and not hypointense on T1-weighted images. Subcortical WML were categorized according to their largest diameter as small (< 3 mm), medium (3-10 mm) or large (> 10 mm). The number of WML was evaluated per size category for

the following locations; frontal, parietal, temporal, occipital, insular, brainstem and cerebellum. Considering them spherical with a fixed diameter per size category, a total approximated volume for subcortical WML was determined.

For evaluation of prevalence and mean volume of WML in each group, correction for inclusion of partial volume was performed as previously described.[2] Briefly, for each participant two small lesions were subtracted from the total number of small WML. This means that women with only two small lesions or less were considered as WML negative. Correction for partial volume was not possible for evaluation of regional distribution since it is not feasible to determine from which brain region(s) the partial volume correction, i.e. two small WML, should be subtracted if a woman demonstrates small WML in more than one region. Therefore, prevalence of WML according to brain region was evaluated in WML positive women only with inclusion of all lesions, i.e. without correction of partial volume.

Data analysis

Descriptive statistics, such as demographic information, are presented as means ± standard deviations for continuous variables and percentages for dichotomous variables.

Demographic data were compared between formerly eclamptic, preeclamptic and control women using ANOVA for normally distributed data, or Kruskall-Wallis test for not normally distributed data. Dichotomous variables, i.e. presence of WML, current hypertension and nulliparity, were compared between the groups using Chi-square, with pair wise Chi-square as post-hoc test. WML volume between formerly (pre)eclamptic and control women was compared using Mann Whitney test.

Differences were considered statistically significant at $P \leq 0.05$. Data analyses were performed using SPSS statistical package for Windows Version 18 (SPSS, Chicago, IL).

Results

Participant characteristics

The inclusion process of the formerly preeclamptic women (n=74) and the women who had normotensive pregnancies (n=75) has been published before.[2] Because the group of formerly eclamptic women has been expanded in the mean time, we now provide detailed description of the recruitment of the entire group of women who suffered eclampsia. In total, 137 formerly eclamptic women were identified. Six of these women were excluded. One because diagnosis of eclampsia could not be confirmed, another because of a history of a cerebrovascular accident and a third due to inability to understand Dutch. In addition, three women had died in the interim; two of whom due to cerebral complications resulting from eclampsia, and one due to cervical cancer. Of the remaining 131 formerly eclamptic subjects, 69 women could be reached and were willing to participate. Six of them were subsequently excluded due to contra-indications for MR scanning. Three of the 69 women who were included in the formerly eclamptic group had not experienced tonic-

clonic seizures. These three, however, were described as having experienced generalized myoclonic twitches while conscious, suggesting cerebral involvement. Therefore, for this study we describe the MRI findings of a total of 63 formerly eclamptic, 74 formerly preeclamptic and 75 parous control participants.

Table 1 shows baseline characteristics of the study participants. Mean age at time of participation was similar for all groups; all groups were in their late thirties. However, elapsed time since index pregnancy was longer for the formerly eclamptic women (7.6 ± 4.7 years) compared to both formerly preeclamptic women (5.2 ± 4.1 years) and parous controls (5.0 ± 3.3 years). Furthermore, the percentage of nulliparous women differed between the groups, with most nulliparous women in the formerly eclamptic group (82.5%), followed by formerly preeclamptic (67.6%) and control women (46.7%). In addition, as expected, birth weight of the child was about 45% lower in the formerly eclamptic and preeclamptic group than the control group. Also estimated gestational age at delivery was more than six weeks shorter in formerly eclamptic and preeclamptic women compared to parous controls.

Current weight was comparable between the three groups. Both systolic and diastolic blood pressures were higher for formerly eclamptic and preeclamptic women compared to the parous controls. Additionally, formerly eclamptic and preeclamptic women were currently hypertensive or using antihypertensive medications four times more often than parous controls.

Subcortical WML

Formerly eclamptic (n=20, 31.7%) and preeclamptic women (n=27, 36.5%) had subcortical WML more often compared to parous controls (n=16, 21.3%). When the presence of WML was compared between the three groups, these differences did not reach significance. However, when formerly eclamptic and preeclamptic women were grouped together, to

Table 1
Participant characteristics

	Controls (n=75)	**Preeclampsia** (n=74)	**Eclampsia** (n=63)
Current age (y)	36.9 ± 6.1	36.6 ± 6.2	38.2 ± 6.3
Elapsed time (y)	5.0 ± 3.3	5.2 ± 4.1	7.6 ± 4.7 *
EGA at delivery (wk)	40.1 ± 1.1 **	33.2 ± 5.1	33.5 ± 4.5
Birth weight of child (g)	3464 ± 462 **	1848 ± 1169	1923 ± 1008
Nulliparous (n)	46.7% (35) §	67.6% (50) †	82.5% (52)
Current SBP (mmHg)	116 ± 12 **	127 ± 12	126 ± 14
Current DBP (mmHg)	74 ± 9 ‡	82 ± 11	79 ± 10
Current hypertension (n)	5.6% (4) ‡	24.3% (18)	20.8% (10)
Current weight (kg)	70.8 ± 10.5	76.1 ± 17.7	73.9 ± 14.9

Data are presented as mean and SD or percentage with the number of women in parentheses.
* P < 0.01 versus controls and preeclampsia, ** P < 0.001 versus preeclampsia and eclampsia, § P < 0.01 versus preeclampsia and P < 0.001 versus eclampsia, † P < 0.05 versus eclampsia, ‡ P < 0.01 versus preeclampsia and P < 0.05 versus eclampsia

enhance statistical power, and compared to parous controls, the (pre)eclamptic group had WML significantly more often than parous controls (35.0 versus 21.3%; $P < 0.05$). In addition, WML were more severe in formerly (pre)eclamptic women than in controls as indicated by a larger WML volume. Mean WML volume for the (pre)eclamptic group was 0.07 mL (range 0.00 – 2.35 mL), and for the control group this was 0.02 mL (range 0.00 – 0.13 mL, $P < 0.05$).

The cerebral regional distribution of lesions in WML positive women was comparable for all groups (Figure 1). In all three groups, the majority of WML was located in the frontal lobes. In the formerly preeclamptic group, all women with WML (n=27) demonstrated these in the frontal lobes. Eighty-five percent (n=17) of the formerly eclamptic women with WML had these lesions in the frontal lobe, for control participants this was 87.5% (n=14). The parietal lobe was the second most affected region by WML, i.e. in 35% of formerly eclamptic (n=7), 29.6% of formerly preeclamptic (n=8) and 25% of control women (n=4). In addition, three formerly preeclamptic women (11.1%) and one control woman (6.3%) demonstrated WML in the temporal lobes. The insular lobes were affected by WML in four formerly eclamptic women (20.0%) and two controls (12.5%). One formerly eclamptic woman (5.0%) had a WML located within the cerebellum. WML in more than one brain region were observed in 40.0% of the formerly eclamptic (n=5), 33.3% of formerly preeclamptic (n=9), and 25.0% of control women (n=4). In all subjects, except for three formerly eclamptic and two controls, WML in the parietal, temporal or insular lobe and cerebellum were accompanied by WML in the frontal lobes. None of the participants showed involvement of the occipital lobe or brainstem.

Periventricular WML

Periventricular WML were present in only one formerly eclamptic woman. She did not demonstrate any subcortical WML. In five formerly preeclamptic women, periventricular WML were observed. These women all demonstrated subcortical WML in addition. None of the control women had periventricular WML. In all women demonstrating periventricular WML, these lesions were considered mild; pencil thin lining surrounding only part of the ventricles.

Infarcts

In the formerly eclamptic group, one woman demonstrated a lacunar infarct and two women had cortical infarcts. Two of these women also demonstrated subcortical WML. In the formerly preeclamptic group, two women had lacunar infarcts and one woman demonstrated a cortical infarct. These three women all had subcortical WML in addition to the infarcts. None of the control women had cerebral infarcts.

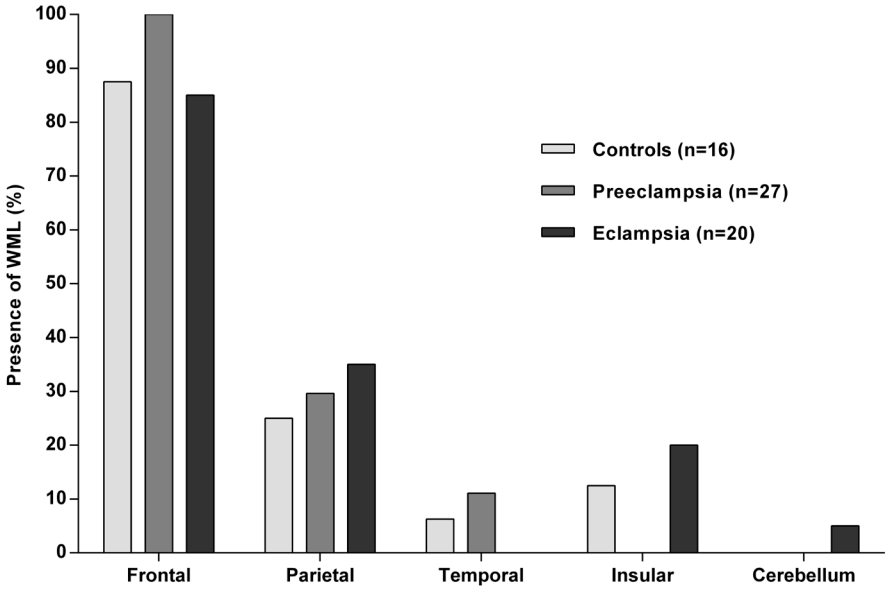

Figure 1. Regional distribution of cerebral WML in WML positive women.
Presence of WML per brain region for WML positive women in the parous control, formerly preeclamptic and formerly eclamptic group. No WML were observed in the occipital lobe and brainstem in any of the groups.

Discussion

Several years following the index pregnancy formerly (pre)eclamptic women had cerebral white matter lesions (WML) more often and more severe compared to parous controls with normotensive pregnancies. The distribution of such WML was similar for formerly eclamptic, formerly preeclamptic and parous control women in that the majority of WML was located in the frontal lobes, followed by the parietal, insular and temporal lobes.

The pathophysiology of WML in formerly (pre)eclamptic women remains speculative and by assessing their distribution we aimed to provide further insight into their development. Previously, we have hypothesized that WML in formerly (pre)eclamptic women may be related to the occurrence of the posterior reversible encephalopathy syndrome (PRES).[1, 2] In PRES, an acute increase in blood pressure may cause loss of cerebral autoregulation and forced dilatation of cerebral arteries, resulting in blood-brain barrier disruption, vasogenic edema formation and the neurologic symptoms of PRES.[23-25] It has been suggested that the vasogenic edema in the acute phase of PRES, when severe enough, may reduce regional cerebral perfusion and blood flow to ischemic levels resulting in areas of cytotoxic edema.[11] These areas may later appear as infarctions or WML on MRI.[11, 12] When reviewing the few existing follow-up studies in obstetric and non-obstetric PRES patients, doubts have been raised concerning the complete reversibility of PRES. There is now evidence for persistent

neuroimaging abnormalities in a substantial part of affected subjects such as infarction, gliosis, hemorrhage, atrophy and laminar necrosis.[14, 15, 18] Studies that longitudinally evaluate eclampsia-related PRES cases with a follow-up between several days and more than seven years, mainly describe presence of WML consistent with gliosis but do not elaborate on their location.[1, 11, 13, 16] We expected that the distribution of WML would be similar to that of the cerebral edema in the acute phase of PRES and suggested a causal relationship between an episode or PRES and the presence of WML. However, while the edema is typically located in the occipito-parietal lobes,[26] the current study demonstrates that only few women had WML in the posterior brain regions. In fact, the majority of WML was located in the frontal lobes. Moreover, this distribution was similar between women who had PRES, i.e. the formerly eclamptic women, and women without PRES, i.e. the parous controls. Therefore, a direct causal relationship between the cerebral edema of PRES and WML in formerly (pre)eclamptic women is less likely.

Alternatively, in the general population the presence of hypertension is associated with cerebral WML,[27-29] which may also explain the higher prevalence of WML in our formerly (pre)eclamptic women. A history of preeclampsia has been associated with an increased risk for cardiovascular disease later in life, including hypertension, ischemic and hemorrhagic stroke, and ischemic heart disease.[3] In this context, the cardiovascular and metabolic demands of normal pregnancy are considered to be a physiologic "stress test" which can provide insight into a woman's future health.[30] Predisposed women fail this "stress test" and develop preeclampsia during pregnancy, thereby revealing their increased risk for cerebro-/cardiovascular disease later in life. In this scheme, an underlying predisposition for cardiovascular disease may result in development of both WML and (pre)eclampsia, without the presence of a direct causal relationship between the two. Our results are in line with this "stress test" theory and epidemiologic findings, with a higher prevalence of current hypertension in formerly (pre)eclamptic women compared to women who had normotensive pregnancies. Moreover, the regional distribution of WML in formerly (pre)eclamptic women is similar to what has been found in other conditions that are associated with cerebro-/cardiovascular disease, such as dementia and migraine.[5, 6, 31] WML in dementia have been related to ischemic changes[32] and migraine is associated with an increased risk for ischemic stroke.[33] Whether a merely subcortical or periventricular WML distribution is related to the pathophysiological mechanism underlying these lesions remains to be elucidated.[22, 34, 35]

In the elderly, the presence and severity of WML is associated with stroke, cognitive decline and dementia.[4] The clinical implications of such lesions in our relatively young cohort of formerly (pre)eclamptic women are currently unknown as reports on WML in this group do not exist. Whether cognitive functioning may sooner or later also be affected in formerly (pre)eclamptic women who have WML, especially since the WML distribution corresponds to that observed in dementia[5,6], is unknown and currently under investigation.

Since a role for PRES in the development of WML cannot be completely ruled out, also clinical follow-up of former PRES patients is of interest. In a rather small percentage of such patients, persistent neurocognitive complaints have been described in addition to abnormalities on cerebral imaging. Self-perceived cognitive functioning is impaired in formerly eclamptic women.[7, 8] Furthermore, cases of diminished objective neurocognitive functioning, visual disturbances and epilepsy have been described after both eclampsia-related as well as non-obstetric PRES.[17-19, 36] However, comprehensive longitudinal studies concerning neurocognitive functioning following PRES are lacking.

A few limitations of the current study should be noted. First, since neuroimaging is not a standard procedure during the acute phase of (pre)eclampsia, the location of cerebral edema in the acute phase of PRES cannot be individually related to the distribution of WML years later. Second, for evaluation of presence of WML per brain region in WML positive women, it was technically not feasible to correct for inclusion of partial volume. Therefore, we might have slightly overestimated the prevalence of WML per brain region.

To our knowledge, this is the first study describing the regional distribution of WML in a relatively large group of formerly (pre)eclamptic women as well as seemingly healthy parous women who never exhibited pregnancy-related hypertension. The regional distribution of WML, mostly in the frontal lobe, may be a reflection of the predisposition for cerebro-/cardiovascular disease in such women. Whether a history of PRES plays an additional role in this relation remains speculative but is less likely. These findings may add to the existing literature concerning the importance of evaluation of cerebro-/cardiovascular risk factors in formerly (pre)eclamptic women. Future research is needed to examine the progress of WML and their clinical implications.

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9

SUMMARY, DISCUSSION, CONCLUSIONS AND FUTURE PERSPECTIVES

Summary

Following the general introduction in **Chapter 1**, this thesis consists of two parts. In **Part One**, animal studies assessing the influence of pregnancy and preeclampsia on the maternal cerebral circulation are presented. **Part Two** of this thesis is focused on visual functioning in both the acute phase of (pre)eclampsia as well as on the long-term follow-up after eclampsia. In addition, neuroimaging data evaluating regional distribution of cerebral lesions following (pre)eclampsia is presented in this second part.

Part I - Animal studies

A previous study has shown that pregnant and nonpregnant animals demonstrated breakthrough of cerebral autoregulation at similar pressures. However, only pregnant animals developed cerebral edema in response to autoregulatory breakthrough. In **Chapter 2**, we investigated the underlying mechanism by which pregnancy may predispose to cerebral edema during acute hypertension. We hypothesized that pregnancy enhances cerebral endothelial cell permeability in response to increased hydrostatic pressure. Therefore, permeability coefficients to Lucifer Yellow (LY) and hydraulic conductivity (L_p; permeability to water), were compared in PCA from nonpregnant and pregnant rats (N=7 for both groups). The permeability coefficient to LY was increased in pregnant versus nonpregnant animals ($P<0.05$), an effect that may predispose the brain to edema formation during acute hypertension. However, pregnancy did not increase L_p, suggesting that pregnancy differentially affects blood-brain barrier permeability to LY and water. To assess whether the increased permeability coefficient to LY was due to elevated estrogen levels during pregnancy, experiments concerning permeability to LY were also performed in ovariectomized (n=6) and estrogen treated ovariectomized rats (n=7). Interestingly, estrogen treatment prevented an increase of the permeability coefficient in ovariectomized animals ($P<0.05$), suggesting that estrogen is protective of BBB permeability and that pregnancy increases BBB permeability by a mechanism other than elevated estrogen.

Cerebral aquaporins (AQPs) may have a role in cerebral edema formation and resolution during pathological states such as eclampsia. Therefore, in **Chapter 3** real-time quantitative polymerase chain reaction was used to assess the expression of AQP1, 4 and 9 in the rat brain during pregnancy and the postpartum state. We found that all three AQPs were expressed in the anterior and posterior cerebrum, cerebellum and brainstem of nonpregnant (N=5), midpregnant (N=5), late-pregnant (N=6) and postpartum (N=5) rats. During pregnancy, the expression of AQP1 and 9 was decreased in the posterior cerebrum and cerebellum. Decreased AQP1 expression in the cerebellum was also observed in postpartum versus nonpregnant animals. In contrast to pregnancy-related decreased expression of AQP1 and 9 in the posterior cerebrum and cerebellum, AQP4 expression was increased in these brain

regions in pregnant compared to nonpregnant animals. However, AQP4 expression in the anterior cerebrum was lower in the postpartum than the nonpregnant state. Since the exact role of AQPs in the brain has not been elucidated yet, the implication of our findings remained so far speculative but may affect edema resolution and/or seizure threshold.

In **Chapter 4,** the influence of preeclampsia, in addition to that of normal pregnancy, on myogenic activity and structure of cerebral resistance arteries was assessed in pregnant rats infused with low-dose endotoxin, which has been shown to be a model for preeclampsia. In this model, endotoxin is considered to induce a low grade systemic inflammatory response, resulting in hypertension, proteinuria, disseminated intravascular coagulation and endothelial activation similar to human preeclampsia. In addition to cerebral arteries, mesenteric resistance arteries were investigated to assess whether these peripheral vessels were similarly affected. Nonpregnant and pregnant rats were infused with either saline (N=9 in both groups) or low-dose endotoxin (N=9 in nonpregnant, N=10 in pregnant group). On day 20 of pregnancy, or 6 days after infusion in nonpregnant rats, the posterior cerebral arteries (PCA) and mesenteric arteries (MA) were used to assess myogenic activity, pressure of forced dilatation and structural properties. In PCA, we found that while normal pregnancy and low-dose endotoxin infusion did not affect structure or myogenic tone within the autoregulatory pressure range, low-dose endotoxin decreased the pressure at which forced dilatation occurred compared to both normal pregnant and nonpregnant rats. These findings suggested that not pregnancy alone, but low-dose endotoxin infusion during pregnancy, which has been shown to be a model for preeclampsia, may predispose the brain to autoregulatory breakthrough and edema formation when blood pressure is increased as seen in eclampsia. In MA, structure was also unaffected by pregnancy or low-dose endotoxin. However, in contrast to PCA, pregnancy alone decreased myogenic tone of MA, with no additional effect of low-dose endotoxin. These different results with respect to myogenic tone in PCA and MA might be explained by the functional difference between the peripheral mesenteric and cerebral vascular bed. The mesenteric arteries show decreased myogenic tone. This could possibly play a role in the decreased peripheral resistance seen during pregnancy. The PCA do not show a decreased myogenic tone during pregnancy. This may be due to the fact that the cerebral vascular system needs to exert a tight autoregulatory blood flow control to prevent ischemia or hyperperfusion. This may be especially important during pregnancy, in the face of the increased plasma volume and cardiac output.

To study whether these changes in PCA function were related to other forms of injury in the brain, we assessed the presence and extent of brain injury in the low-dose endotoxin-infused pregnant rat model by measuring the presence of the neuroinflammatory marker

S100B in the brain and the peripheral circulation in **Chapter 5**. No difference in S100B expression in the brain was found between nonpregnant saline-treated (N=5), nonpregnant endotoxin-treated (N=7), pregnant saline-treated (N=7) and pregnant endotoxin-treated (N=7) rats. Furthermore, plasma levels of S100B were similar in pregnant endotoxin- and saline-treated rats. From these results, we concluded that pregnancy nor low-dose endotoxin (experimental preeclampsia) affected S100B brain expression and plasma levels suggesting the absence of brain injury in this model.

Part II – Human studies

Chapter 6 reviews the literature concerning visual disturbances in patients with preeclampsia, eclampsia and HELLP-syndrome. Visual disturbance is a relatively common phenomenon in (pre)eclamptic patients, therefore, obstetricians may encounter women with serious, and sometimes debilitating, pathology of the visual pathways. Several ophthalmologic entities are associated with (pre)eclampsia, which include cortical blindness, serous retinal detachment, Purtscher-like retinopathy, central retinal vein occlusions, and retinal or vitreous hemorrhages. Ensuing visual symptoms experienced by these patients are blurry vision, diplopia, amaurosis fugax, photopsia and scotomata, including homonymous hemianopsia. In general, aside from lowering the blood pressure and preventing (further) seizures, no specific therapy seems indicated for (pre)eclamptic women who experience visual disturbances. In most cases, visual acuity returns back to normal after the preeclamptic pregnancy. However, permanent visual impairment in (pre)eclamptic women has incidentally been described and related to persistent cerebral lesions consistent with infarction and/or retinal abnormalities.

As brain white matter lesions have been described in formerly (pre)eclamptic women, and might be related to visual impairment, especially when located in the cerebral visual areas, in **Chapter 7** we sought to determine whether formerly eclamptic women experience lower vision-related quality of life. Therefore, formerly eclamptic women (N=46) and controls following normotensive pregnancies (N=47) completed the vision-related quality of life questionnaire (NEI-VFQ-39/NL). Composite scores and 4 out of 12 subscale scores of the NEI-VFQ-39/NL, in specific, general health, vision-related social functioning, driving and peripheral vision, were significantly lower in formerly eclamptic women compared to controls ($P<0.01$ for composite scores). To assess whether these lower scores in formerly eclamptic women could be explained by visual field loss due to white matter lesions (WML), these women underwent formal visual field assessment (N=43). All visual fields appeared intact, suggesting that lower vision-related quality of life scores in formerly eclamptic women were independent of visual field loss. However, WML were present in 35.7% of formerly eclamptic women who underwent MRI (N=42) and these WML were associated

with the lower NEI-VFQ-39/NL composite scores and 4 out of 12 subscale scores, in specific, general vision, near activities, vision-related role function and peripheral vision ($P<0.05$ for composite scores). Therefore, these lower composite and subscale scores in the subgroup of formerly eclamptic women with WML might be related to impaired higher-order visual functioning due to the presence of WML in cerebral areas involved in these functions.

In **Chapter 8** we aimed to gain further insight into the possible consequences and pathogenesis of the cerebral WML following eclampsia described in the previous chapter. We assessed the regional distribution of these lesions on MRI scan in women years following eclampsia (N=63) and normotensive pregnancies (N=75) and also included formerly preeclamptic women (N=74). We found, in accordance with previous studies, that formerly (pre)eclamptic women more often demonstrated WML than women who had normotensive pregnancies (35.0% versus 21.3%; $P<0.05$). Moreover, WML in the (pre)eclamptic group were more severe than in the group with normotensive pregnancies (0.07 versus 0.02 mL; $P<0.05$). In all groups, the majority of WML was located in the frontal lobes, and to a lesser extent in the parietal, insular and temporal lobes. This led to the conclusion that the regional WML distribution was similar for all three groups. Interestingly, this distribution corresponded to the distribution observed in conditions that are associated with cerebro-/cardiovascular disease, such as migraine and dementia. Therefore, WML in formerly (pre)eclamptic women may be a reflection of the predisposition for cerebro-/cardiovascular disease in these women. These findings may add to the existing literature concerning the importance of evaluation of cerebro-/cardiovascular risk factors in formerly (pre)eclamptic women. Furthermore, it has been suggested that a history of the posterior reversible encephalopathy syndrome (PRES), which is considered the underlying cause of the neurological symptoms in the acute phase of (pre)eclampsia, may play an additional role in the development of WML. Although this seems less likely in view of the WML distribution, future studies should address the possible relation between PRES and WML.

General discussion

Pathophysiology of eclampsia

The brain during pregnancy

Although the exact pathophysiology of eclampsia still remains to be elucidated, the general thought is that eclampsia develops in preeclamptic women when, in the presence of endothelial dysfunction, the blood pressure rises beyond the upper limit of cerebral autoregulation. Interestingly, however, eclamptic women often do not reach blood pressure values generally known to be in this upper limit range.[1, 2] This suggests that pregnancy, and preeclampsia in particular, somehow predisposes the brain to autoregulatory breakthrough. To investigate this, animal studies are needed and such studies from our

group have therefore focused on the question whether pregnancy induces a leftward shift of the cerebral autoregulation curve. If such a shift occurs, lower blood pressures may induce loss of autoregulation. In our ex vivo study, we were unable to demonstrate an effect of pregnancy on the blood pressure at which forced dilatation occurred in posterior cerebral arteries of Wistar rats, suggesting no leftward shift of the upper limit of autoregulation (**Chapter 4**). However, this may be strain dependent since in Sprague Dawley rats a decreased pressure at which forced dilatation occurred was demonstrated during pregnancy.[3] These above mentioned studies evaluated aspects of autoregulation in isolated arteries which lack the influence of several factors involved in autoregulation in vivo. Examples of such factors are perivascular innervation, circulating substances in blood and shear stress due to blood flow.[4, 5] In vivo experiments in Sprague Dawley rats, in fact, demonstrated forced dilatation at similar blood pressures in nonpregnant and pregnant animals.[6] Interestingly, in this latter study, while the pressure at which breakthrough of autoregulation occurred appeared to be similar in pregnant and nonpregnant rats, only pregnant rats developed brain edema in response to this breakthrough.[6] Furthermore, blood-brain barrier permeability for both 70,000 MW dextran and sodium fluorescein was increased in pregnant rats during a period of acute hypertension compared to nonpregnant rats.[7] These findings suggest that it may not be a shift of the autoregulatory curve but rather an increased susceptibility for blood-brain barrier disruption, and edema formation, that underlie the cerebrovascular adaptation of pregnancy. Several mechanisms may underlie this susceptibility during pregnancy and have been the focus of studies in the Cipolla lab.

First, pregnancy seems to affect barrier properties of the cerebral endothelium thereby enhancing blood-brain barrier permeability. In rat posterior cerebral arteries, both increased permeability to dextran[8] and Lucifer Yellow (**Chapter 2**) and enhanced pinocytosis (i.e. transcellular transport) were observed in response to increased pressure during pregnancy.[8] However, permeability to water (i.e. hydraulic conductivity) appeared unaffected (**Chapter 2**). This differential effect of pregnancy on permeability to water versus solutes such as Lucifer Yellow and dextran suggests that pregnancy enhances transcellular transport without increasing hydraulic conductivity. The implications of this differential effect as well as the exact mechanistic basis of pregnancy-induced increased blood-brain barrier permeability remain so far elusive. The elevated estrogen levels during pregnancy appeared unrelated to this enhanced permeability (**Chapter 2**). Future studies should focus on other possible candidates contributing to or responsible for inducing increased arterial blood-brain barrier permeability during pregnancy, including progesterone or other pregnancy hormones, and angiogenic factors.

Secondly, aquaporins, in particular aquaporin 4 as the most abundant cerebral aquaporin, have been suggested to play a role in the vulnerability towards cerebral edema formation during pregnancy.[9] Given the localization of aquaporin 4 in the astrocytic

endfeet bordering the cerebral vasculature,[10] this aquaporin may have a function in uptake of water that has entered the brain after blood-brain barrier disruption. In this scheme, aquaporin 4 may contribute to resolution of cerebral vasogenic edema. This is supported by studies using a freeze injury model of vasogenic edema, which showed increased brain water content in aquaporin 4 knockout mice compared to wild type mice.[11, 12] A role in vasogenic edema formation seems less likely since aquaporin expression in the cerebral endothelium is doubtful.[10, 13-15] As pregnant animals are more sensitive to formation of edema, one would hypothesize that the expression of aquaporin 4 would be decreased in pregnant animals. However, in contrast, we found increased expression mRNA of aquaporin 4 in the posterior cerebrum and the cerebellum in pregnant versus nonpregnant rats (**Chapter 3**). In addition, aquaporin 4 protein expression was shown to be increased during pregnancy.[16] The implications of these pregnancy-induced increases in cerebral aquaporin 4 expression remain so far speculative. For example, the increased expression of aquaporin 4 may represent a protective mechanism against the vulnerability of this brain region for vasogenic edema by its role in edema resolution. Alternatively, this altered aquaporin 4 expression may influence susceptibility to seizures during pregnancy since mice lacking aquaporin 4 had higher seizure threshold.[17]

Finally, a pregnancy-induced decrease in cerebrovascular resistance during pregnancy with subsequent increased hydrostatic pressure on the downstream microcirculation may enhance blood-brain barrier permeability. This is illustrated by the fact that pregnancy induced outward remodeling of rat penetrating brain arterioles, which appeared associated with decreased cerebrovascular resistance and hyperperfusion during acute hypertension, compared to the nonpregnant state.[7] Furthermore, increased cerebral capillary density during pregnancy may also result in diminished cerebrovascular resistance. In situations accompanied by hypertension, this may lead to hyperperfusion and increased pressure on the downstream microcirculation.[7] From this study, it therefore appeared that pregnancy may indeed enhance vulnerability of the blood-brain barrier by decreased cerebrovascular resistance when blood pressure is increased.

While the above mentioned studies have focused on cerebral arteries, the primary site of blood-brain barrier disruption might not reside in the cerebrovasculature's arterial tree. In fact, blood-brain barrier disruption during acute hypertension is primarily described in cerebral veins,[18, 19] making the influence of pregnancy on venous blood-brain barrier permeability of interest. A recent study showed that nonpregnant rat cerebral veins that were exposed to plasma from pregnant women had increased blood-brain barrier permeability compared to veins that were not exposed to plasma.[20] Although this study may suggest that pregnancy-related circulating plasma factors may increase venous permeability, another study in cerebral veins demonstrated no difference in permeability when nonpregnant veins were perfused with nonpregnant plasma and compared to pregnant veins perfused with pregnant plasma.[21] In fact, this latter study showed that

pregnancy protects against increases in blood-brain barrier permeability related to vascular endothelial growth factor (VEGF) or placental growth factor (PLGF) by increased levels of soluble fms-like tyrosine kinase-1 (sFlt-1).[21] Additional future studies are needed to further elucidate the influence of pregnancy and circulating pregnancy factors on cerebral venous blood-brain barrier permeability.

In conclusion, although it has been the focus of several studies, a complete picture of the pregnancy-induced adaptation of the cerebral vasculature is lacking. Results from the above mentioned animal studies in pregnancy suggest a predilection for development of cerebral edema during, possibly even mild, increases in blood pressure. While a pregnancy-related shift of the cerebral autoregulation curve seems less likely, other mechanisms such as a decrease in cerebrovascular resistance and/or enhanced blood-brain barrier permeability during pregnancy may be in place. To further elucidate such adaptations, more studies focusing on cerebral autoregulation and the blood-brain barrier in relation to pregnancy are required.

The brain during preeclampsia

Although studies in normal pregnancy may certainly add to our understanding of the cerebrovascular pathophysiology of preeclampsia, the influence of preeclampsia on the cerebral circulation is of particular interest. We raised the question whether preeclampsia further increases the tendency for cerebral edema formation, in addition to the effects of pregnancy per se. In this context, we hypothesized that preeclampsia decreases the blood pressure at which loss of autoregulation occurred (**Chapter 4**), thereby increasing the likelihood of edema formation at lower blood pressures. We used the low-dose endotoxin-infused pregnant rat as experimental model for preeclampsia. This model is characterized by hypertension, proteinuria, intravascular disseminated coagulation, generalized activation of the inflammatory response as well as endothelial activation.[22-25] The development of the preeclamptic-like syndrome in this model is considered to result from a systemic inflammatory response induced by endotoxin.[23, 25, 26] Moreover, the syndrome is exclusively seen in pregnant animals, i.e. identically treated non-pregnant rats did not show the above mentioned signs.[22-24] Given the severity of these symptoms and the limited effects on the fetuses, this model is considered a model of mild preeclampsia.[22, 23] Even in this mild model of preeclampsia, we found that posterior cerebral arteries underwent forced dilatation at lower pressures compared to healthy pregnant rats. The mechanistic basis for forced dilatation at lower blood pressure during preeclampsia is so far unclear but might be related to increased sensitivity of cerebral arteries to nitric oxide, since it was recently shown that cerebral arteries from low-dose endotoxin-treated pregnant rats showed enhanced sensitivity to the dilatory effect of a nitric oxide donor[27] and increased vascular inducible nitric oxide synthase,[27] which might both explain the forced dilatation

at lower pressures. Whether similar changes occurred in the rats in our study remains to be studied, as the model of Cipolla et al, is different from our model, since a slightly higher dose of endotoxin was infused under isoflurane anesthesia, rather than under awake conditions.[27] In addition, we did not measure blood pressure and thus direct comparisons cannot be made. The fact that even mild preeclampsia in our study decreased the pressure of forced dilatation, may suggest that in more severe models of preeclampsia, the pressure at which forced dilatation occurs might even be further decreased, thereby enhancing the predisposition for loss of autoregulation and cerebral edema formation.

In addition to a decreased pressure of forced dilatation in experimental preeclampsia, studies in hypertensive pregnancy models have shown that pregnancy prevents, and even reverses, protective remodeling of posterior cerebral arteries in response to hypertension.[28-30] Since hypertensive remodeling attenuates the increased pressure on the downstream vasculature, it is considered a protective mechanism against the effects of an episode of acute hypertension.[31] Lack of this remodeling may, therefore, lead to earlier forced dilatation at lower pressures and increased vulnerability of the blood-brain barrier to disruption. Our findings of lack of remodeling of cerebral arteries in experimental preeclamptic rats are in line with these previous findings of prevention of hypertensive remodeling during pregnancy (**Chapter 4**). Unfortunately, it was technically not possible to measure blood pressure in the rats in our study. From previous studies, we expect a mild rise in blood pressure (from about 110-120 to 130-140 mmHg), mainly in the last few days of pregnancy.[22, 24, 32] The lack of remodeling could be due to this mild hypertension. Alternatively, the lack of blood pressure data does also leave the possibility that our rats did not reach hypertensive values, which may also explain lack of hypertensive remodeling.

In addition to these altered features pertaining to autoregulation, barrier properties of the cerebral endothelium seem also further impaired during preeclampsia. Exposure of cerebral rat veins to human preeclamptic plasma significantly increased blood-brain barrier permeability compared to exposure to plasma of normotensive pregnant women.[20] Whether this is also true for arteries, is unknown. While vascular endothelial growth factor (VEGF) is held responsible for this increase,[21] several other factors may be involved in blood-brain barrier permeability during (pre)eclampsia. For example, oxidative stress, histamine, nitric oxide, bradykinin and pro-inflammatory cytokines, such as tumor necrosis factor, interleukins and growth factors, all seem to increase blood-brain barrier permeability.[33] Since preeclampsia is also associated with oxidative stress and increased circulating proinflammatory cytokines in association with endothelial dysfunction, these factors might as well increase blood-brain barrier permeability changes during preeclampsia.[34, 35]

Results from the above mentioned animal studies suggest that preeclampsia, in addition to the suggested pregnancy-induced adaptation, may further sensitize the brain to cerebral edema formation in face of increased blood pressure. They suggest that both a leftward shift of the cerebral autoregulation curve during preeclampsia as well

as changes in blood-brain barrier properties might play a role. However, these studies should just be considered the first pieces of the puzzle. Much more research is needed to unravel the influence of preeclampsia on the brain so that eventually brain involvement in preeclampsia can be prevented and/or adequately treated. In this light, studies focusing on neuroinflammatory markers of blood-brain barrier disruption are highly interesting. Such markers might be used to diagnose brain involvement in preeclamptic women in an early stage, during which clinical signs of brain involvement are still absent or indistinct. Use of these markers, such as S100B, glial fibrillary acidic protein (GFAP) and neuron-specific enolase (NSE), seems promising in diagnosing brain damage and blood-brain barrier dysfunction in various neuropathologic states. Examples include cerebral hemorrhage, ischemic stroke, traumatic injury and iatrogenic blood-brain barrier disruption.[36-38] Since these markers might also be useful in recognizing brain involvement in preeclamptic patients, we aimed to gain insight in S100B brain expression and plasma levels during experimental preeclampsia in de rat. Using the low-dose endotoxin-treated rat as model for preeclampsia, we found no effects of preeclampsia on both S100B brain expression and plasma levels (**Chapter 5**), suggesting that experimental preeclampsia did not induce brain injury. However, since women with severe preeclampsia are more at risk for cerebral involvement than women with mild preeclampsia,[39] our findings might be explained by the fact that we used a model for mild preeclampsia. It is therefore possible that S100B brain expression and plasma levels are increased in animal models of more severe preeclampsia.

Long-term consequences of (pre)eclampsia

Neuroimaging findings

While the obstetrical literature and textbooks used to state that (pre)eclamptic women could expect full recovery, the persistent complaints by such women suggests otherwise. Years following (pre)eclampsia a substantial number of these women report impaired physical and mental well being.[40, 41] Examples of common complaints include headache, visual disturbances, tiredness and loss of concentration or memory.[40, 41] In addition, persistent abnormalities on cerebral imaging have been observed. Both formerly preeclamptic and eclamptic women demonstrate cerebral white matter lesions more often than women who had normotensive pregnancies (**Chapter 8**).[42, 43] These reports hypothesized that such lesions were related to an episode of the posterior reversible encephalopathy syndrome (PRES), which is considered the underlying cause of the cerebral edema and neurological symptoms in eclamptic as well as some preeclamptic patients.[2, 9] This syndrome can also be encountered in several other patient categories, for example in patients with renal inflammatory conditions, chemotherapy and immunosuppression after organ transplantation.[44, 45] Although follow-up studies in such non-obstetric PRES patients are largely lacking, the few available results have also shown persistent neuroimaging abnormalities in a substantial part of these patients, including white matter lesions.[45-47]

Subsequently, questions concerning the pathogenesis as well as the clinical implications of these lesions arose, which will be discussed below.

While previous studies were not designed to provide insight into the pathogenesis of white matter lesions, it was deemed plausible that the vasogenic edema in PRES was responsible for inducing white matter lesions. With regards to this, vasogenic edema may progress to such an extent that regional blood flow and cerebral perfusion decreases to ischemic levels leading to areas of cytotoxic edema and the development of white matter lesions in the long-term.[48, 49] The fact that more severe eclampsia, in terms of number of seizures, appeared related to a higher lesion load supported this hypothesis.[42] However, with our demonstration of a mainly frontal localization of white matter lesions (**Chapter 8**), a direct causal relationship between the vasogenic edema of PRES and white matter lesions in the long-term seems doubtful since the edema in PRES is mainly located in the posterior brain regions.[44] The distribution rather suggests that these lesions are an expression of the predisposition of formerly (pre)eclamptic women for cerebro-/cardiovascular disease. In accordance with this hypothesis, lesion distribution in conditions commonly associated with cerebro-/cardiovascular disease, such as migraine and vascular dementia, appear similar to that found following (pre)eclampsia.[50, 51]

As stated, epidemiologic research in recent years has revealed an association between a history of preeclampsia and cerebro-/cardiovascular disease later in life.[52] Formerly preeclamptic women have an increased risk for development of systemic hypertension, ischemic and hemorrhagic stroke, ischemic heart disease and venous thromboembolism. Furthermore, these women have unfavourable cardiovascular risk profiles.[53, 54] Though the mechanistic basis of the increased risk for cerebro-/cardiovascular disease in women who had preeclampsia remains elusive so far, many risk factors are shared by both conditions,[53, 54] suggesting that both conditions are an expression of the same underlying predisposition. Examples of such common risk factors are hypertension, insulin resistance, hyperlipidemia and obesity.[53, 54] In this context, the cardiovascular and metabolic demands of normal pregnancy are considered to be a physiologic "stress test" which can provide insight into a woman's future health.[55] Predisposed women fail this "stress test" and develop preeclampsia during pregnancy, thereby revealing their increased risk for cerebro-/cardiovascular disease later in life. In this scheme, an underlying predisposition for future cerebro-/cardiovascular disease may result in development of both cerebral white matter lesions and (pre)eclampsia earlier in life, without the obvious presence of a definite causal relationship between the two. An episode of PRES may in this light be seen as the death blow.

Clinical implications of cerebral white matter lesions

The clinical implications of cerebral white matter lesions in women who previously had (pre)eclampsia are mostly hypothetic. Few cases have been published showing impaired

neurocognitive functioning and visual disturbances on clinical follow-up after pregnancy-related and non-obstetric PRES.[56, 57] Part of these clinical sequelae was thought to be related to permanent cerebral lesions. However, the exact relationship between neuroimaging findings and clinical symptoms after PRES remains largely unknown.

White matter lesions are not only observed in former PRES patients. One-fifth of our healthy parous control women also demonstrated such lesions (**Chapter 8**). This is in line with a previous study which also reported a rather high prevalence of white matter lesions in relatively young healthy cohorts.[58] The exact clinical importance of this lesion burden in a young cohort, as in our study, is not clear and may be related to the normal process of ageing. Concerning, though, is the fact that in the elderly there is evidence that the presence, and particularly the severity, of white matter lesions are risk factors for the development of cognitive impairment, vascular dementia, Alzheimer's disease and stroke.[59] One could therefore hypothesize that white matter lesions in our formerly (pre)eclamptic women may also be associated with impairment of cognitive functioning and might even predispose to dementia. Even though this remains rather speculative as of 2012, it is of particular interest since preeclampsia and Alzheimer's disease appear to share (epi)genetic features, namely expression of the STOX1 gene.[60] In preeclampsia, this placentally-expressed gene has been proposed to be involved in trophoblast dysfunction, whereas in Alzheimer's disease cerebral STOX1 expression may have a role in amyloid deposition, a key event in the pathogenesis of Alzheimer's disease.[61, 62] However, sizeable studies concerning the causal relationship between white matter lesions and impaired cognitive functioning are not yet available. Small studies, however, indicate that self-reported cognitive functioning seems lower in women who had eclampsia,[63] while we have now shown that white matter lesions in formerly eclamptic women seemed associated with decreased vision-related quality of life (**Chapter 7**). The latter might be related to impaired higher-order neurocognitive functions involved in vision since visual fields of these women were intact. Studies regarding objective neurocognitive functioning following (pre)eclampsia have so far been inconclusive.[64-66]

Further studies regarding cognitive functioning following (pre)eclampsia and its relationship with white matter lesions are required to answer the ominous question whether a history of (pre)eclampsia might be related to cognitive impairment and even dementia later in life. But, since white matter lesions following (pre)eclampsia may be considered an expression of a predisposition to future cerebro-/cardiovascular disease, this stresses the importance of evaluation of cerebro-/cardiovascular risk factors in such women. In this context, postpartum lifestyle interventions seem hope giving, since such a program has been proven successful in reducing several risk factors for developing cerebro-/cardiovascular disease.[67]

Final conclusions

Animal studies in this thesis found that pregnancy increased blood-brain barrier permeability of cerebral arteries to Lucifer Yellow in response to rising pressure. This increase in blood-brain barrier permeability may predispose to cerebral edema formation during acute hypertension and appeared unrelated to elevated estrogen levels during pregnancy. The mechanism underlying the increase in blood-brain barrier permeability should therefore be the focus of future studies. Furthermore, pregnancy and the postpartum state induce changes in cerebral aquaporin expression. While these changes may relate to altered edema resolution and seizure threshold, the implication remains to be elucidated. In addition to these pregnancy-related adaptations of the cerebrovasculature contributing to vulnerability for cerebral edema formation during acute hypertension, preeclampsia seemed to further increase this vulnerability. In an animal model of preeclampsia, posterior cerebral arteries underwent forced dilatation at lower pressure compared to normal pregnancy, suggesting a possible leftward shift of the upper limit of autoregulation. Despite these suggested effects on autoregulation, preeclamptic animals did not demonstrate signs of brain injury as assessed by the neuroinflammatory marker S100B, which might be explained by the fact that we used a model for mild preeclampsia. In conclusion, these studies have expanded our current knowledge about pregnancy-related adaptation of the cerebral vasculature and how such adaptation may enhance the vulnerability of the brain to cerebral edema formation during acute hypertension, as in eclampsia. Additionally, this thesis suggests that preeclampsia may even further increase this vulnerability.

Human studies in this thesis demonstrated the presence of long-term brain involvement following (pre)eclampsia. Formerly eclamptic women reported lower vision-related quality of life several years following eclampsia, which appeared at least partly related to the presence of cerebral white matter lesions. Since these women had intact visual fields, decreased vision-related quality of life compared to women following a normotensive pregnancy might be due to impairment of higher-order visual functions. Furthermore, formerly (pre)eclamptic women demonstrate a higher prevalence of cerebral white matter lesions than women who had normotensive pregnancies. The regional distribution of these lesions suggests a relationship with a propensity towards cerebro-/cardiovascular disease rather than a direct causal relation with a prior episode of PRES. These studies add to the existing evidence that (pre)eclampsia should not be conceptualized as a completely reversible condition, from which women can expect full recovery. In addition, they emphasize the importance of evaluation and reduction of cerebro-/cardiovascular risk factors in such women.

Future perspectives

Cerebrovascular changes in pregnancy and preeclampsia

Several animal studies have focused on the adaptation of the brain to pregnancy. However, how pregnancy affects cerebral autoregulation and the blood-brain barrier has still not completely been unraveled. Future studies in both animal models and the human should gain further insight into pregnancy-induced adaptation of both the arterial and venous circulations of the brain. Moreover, studies evaluating whether and how preeclampsia additionally affects the cerebral circulation should be continued. Such studies should make use of different experimental animal models of preeclampsia, such as models based on reduced uterine perfusion pressure (RUPP) or soluble fms-like tyrosine kinase-1 (sFlt1) infusion.[68] This will not only answer the question whether study results are model-dependent but may also give insight into cerebrovascular functioning in relation to severity of preeclampsia. In studies using such models, it would be highly interesting to focus on the underlying mechanism by which pregnancy and preeclampsia enhance vulnerability of the blood-brain barrier. While estrogen seemed unrelated to increased blood-brain barrier permeability during pregnancy, the role of several other factors should be assessed. For example, effects of endothelial dysfunction and the various circulating factors, such as pro-inflammatory cytokines and (anti)angiogenic substances, pertaining to this preeclampsia-related vulnerability should be assessed. When such mechanisms become unraveled, the next step would be to shift the focus towards studies assessing methods to protect the blood-brain barrier.

Recognition of impending brain involvement in preeclampsia

To aid in the diagnosis of (impending) cerebral involvement during preeclampsia, future studies should be directed towards the identification and usefulness of neuroinflammatory markers in preeclampsia. These markers, such as S100B and neuron-specific enolase (NSE), may be increased in serum and/or cerebrospinal fluid before clinical symptoms of brain involvement become apparent. Early recognition of cerebral involvement in preeclamptic women may eventually lead to prevention of progression to eclampsia/PRES or other devastating complications such as hemorrhage. Therefore, both animal and clinical studies in healthy pregnant and preeclamptic subjects should be designed to determine levels of several neuroinflammatory markers in serum and cerebrospinal fluid. These studies should compare levels between normal pregnant and preeclamptic subjects and assess the relation between these levels and cerebral involvement.

Long-term consequences of (pre)eclampsia

While impaired cognitive functioning has been suggested following (pre)eclampsia, objective neurocognitive studies are needed to further evaluate cognitive functioning in

such women and to assess whether higher-order visual functions are impaired. The presence of a relation between results from these neurocognitive tests and cerebral neuroimaging findings should be investigated. In these analyses not only presence and severity of cerebral lesions should be taken into account, but also their regional distribution as this may give clues towards their clinical sequelae.

To further elucidate the role of PRES in the pathogenesis of cerebral white matter lesions and long-term consequences following (pre)eclampsia, additional studies are warranted that include patient categories with non-obstetric PRES. Neuroimaging and neurocognitive follow-up data from these non-obstetric PRES patients should be compared to data from (pre)eclamptic PRES patients.

Concerning the future clinical implications of cerebral white matter lesions in our cohorts of formerly (pre)eclamptic women, studies with extensive follow-up periods should be designed. These studies may give answer to the questions whether these women are at increased risk for developing future cognitive impairment, or even dementia, and cerebro-/cardiovascular disease and whether this is related to white matter lesions earlier in life. Since women within this group are probably not homogeneous with respect to these future sequelae, an emphasis should be placed on the identification of women with the highest risk for developing cerebro-/cardiovascular disease. In addition to long-term follow-up studies, retrospective studies in cohorts of women with dementia may give insight in the possible relation with preeclampsia earlier in life.

Interventions for formerly (pre)eclamptic women

In view of the increased risk for future cerebro-/cardiovascular disease in formerly (pre)eclamptic women, studies are needed that focus on the development and establishment of effective and feasible lifestyle intervention programs for formerly (pre)eclamptic women. Such interventions may reduce risk factors, such as overweight, cholesterol levels and blood pressure, in those women who are prone to developing cerebro-/cardiovascular disease later in life. In addition to interventions addressing cardiovascular risk factors, attention should be paid towards investigating therapeutic and counseling modalities for a subgroup of formerly eclamptic women who experience impaired cognitive functioning.

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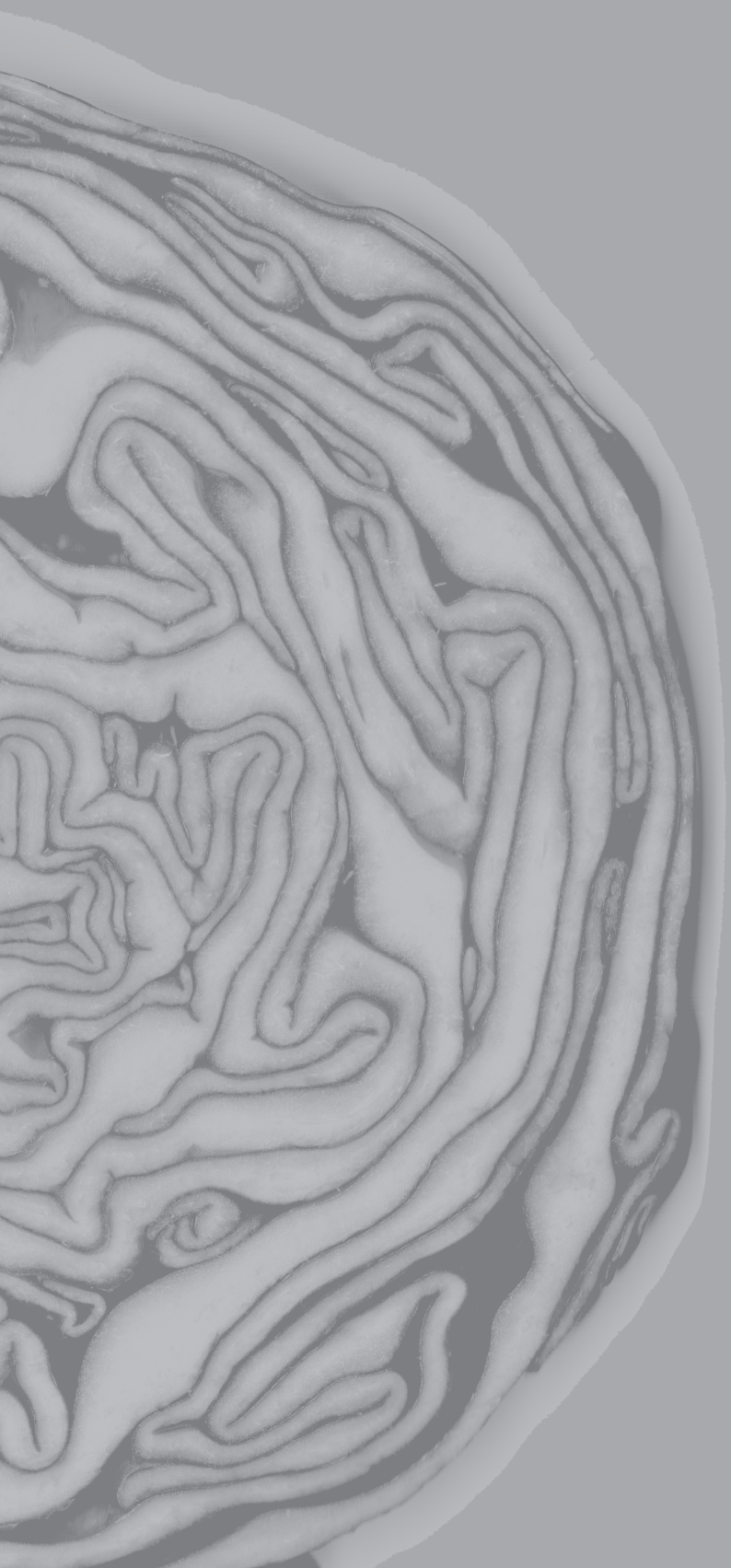


NEDERLANDSE SAMENVATTING

Pre-eclampsie is een zwangerschapsgerelateerde aandoening die optreedt bij 5 tot 7% van de zwangerschappen. De aandoening is gedefinieerd als het optreden van hypertensie en proteïnurie tijdens de tweede helft van de zwangerschap bij een voorheen normotensieve vrouw. Pre-eclampsie is een systemische aandoening waarbij onder andere de lever en nieren betrokken kunnen raken. Ook de hersenen kunnen aangedaan zijn in de vorm van eclampsie, het optreden van tonisch-clonische insulten bij een vrouw met pre-eclampsie zonder andere aanwijsbare oorzaak. Naast eclamptische insulten kunnen andere neurologische verschijnselen voorkomen, waaronder hoofdpijn, misselijkheid en braken, en visusstoornissen. Hoewel hersenbetrokkenheid bij pre-eclampsie weinig voorkomt, is het verantwoordelijk voor een substantieel deel van de maternale sterfte in de westerse wereld en zo ook in Nederland.

Om eclampsie adequaat te kunnen voorkomen, behandelen, en eventuele langetermijngevolgen te begrijpen is kennis over het ontstaan van eclampsie essentieel. In **Hoofdstuk 1** wordt ingegaan op de pathofysiologie van eclampsie. Omdat de cerebrale circulatie hierbij een cruciale rol speelt, worden in dit hoofdstuk verschillende aspecten van de cerebrale circulatie besproken, te weten cerebrale autoregulatie en de bloed-hersenbarrière. Deze cerebrale autoregulatie, middels aanpassing van de diameter van de bloedvaten, zorgt ervoor dat de cerebrale bloeddoorstroming gelijk blijft ondanks veranderingen in bloeddruk. Wanneer de bloeddruk echter boven de maximale grens van de cerebrale autoregulatie stijgt, zoals bij eclampsie wordt aangenomen het geval te zijn, ontstaat er geforceerde dilatatie van de bloedvaten. De huidige opvatting over de pathofysiologie van eclampsie is dan ook dat een acute bloeddrukstijging, in combinatie met endotheeldysfunctie, leidt tot geforceerde dilatatie van de cerebrale vasculatuur. Dit resulteert vervolgens in cerebrale hyperperfusie, gevolgd door beschadiging van de bloed-hersenbarrière. Hierdoor kunnen water, eiwitten en electrolyten uit de bloedvaten treden en wordt vasogeen oedeem gevormd. Dit oedeem veroorzaakt vervolgens het zogenaamde ‘posterior reversible encephalopathy syndrome’ (PRES). Het is echter nog grotendeels onbekend waarom de hersenen tijdens zwangerschap, en pre-eclampsie in het bijzonder, vatbaar zijn voor het ontwikkelen van PRES/eclampsie. In **Deel 1** van dit proefschrift wordt daarom in diermodellen gekeken naar de invloed van zwangerschap en pre-eclampsie op de cerebrale circulatie.

Tot voor kort werd gedacht dat in geval van eclampsie, wanneer de bloeddruk normaliseert, het hersenoedeem verdwijnt en de patiënte volledig herstelt. Recentelijk is men dit volledige herstel echter in twijfel gaan trekken, zoals in **Hoofdstuk 1** wordt besproken. Veel vrouwen melden neurocognitieve klachten na het doormaken van eclampsie, waaronder problemen met geheugen en concentratie. Daarnaast vertonen zij jaren na het doormaken van eclampsie vaker wittestoflaesies op MRI scans van de hersenen. In **Deel 2** van dit proefschrift wordt gekeken naar de regionale verdeling van deze laesies en mogelijke langetermijngevolgen op het visueel functioneren. Daarnaast worden in deel 2 visusstoornissen in de acute fase van eclampsie besproken.

Deel I - Dierexperimentele studies

Uit eerder onderzoek is gebleken dat bij zwangere en niet-zwangere ratten doorbraak van de cerebrale autoregulatie plaatsvond bij vergelijkbare drukken. Echter, alleen zwangere ratten ontwikkelden cerebraal oedeem in respons op deze doorbraak. In **Hoofdstuk 2** wordt gekeken naar het onderliggende mechanisme waarop zwangerschap de hersenen vatbaarder maakt voor het ontwikkelen van oedeem tijdens acute hypertensie. De hypothese was dat de permeabiliteit van het cerebrale endotheel in respons op hydrostatische druk toeneemt tijdens zwangerschap. Om dit te onderzoeken werden de permeabiliteitscoëfficiënten van Lucifer Yellow (LY) en de hydraulische conductiviteit (L_p; permeabiliteit voor water) in de arteria cerebri posterior (ACP) van niet-zwangere en zwangere ratten vergeleken (n=7 in beide groepen). De permeabiliteitscoëfficiënt van LY was toegenomen in zwangere versus niet-zwangere proefdieren ($P<0.05$). Dit zou de hersenen mogelijk vatbaarder kunnen maken voor oedeemvorming tijdens acute hypertensie. Tijdens zwangerschap nam L_p echter niet toe, wat suggereert dat zwangerschap een verschillend effect heeft op bloed-hersenbarrière permeabiliteit voor LY en water.

Om te onderzoeken of de toegenomen permeabiliteitscoëfficiënt voor LY een gevolg was van verhoogde oestrogeenspiegels tijdens zwangerschap, werd de permeabiliteit voor LY tevens onderzocht in ratten die een ovariëctomie hadden ondergaan en vervolgens behandeld werden met oestrogeen (n=7) of placebo (n=6). Hierbij bleek dat oestrogeenbehandeling toename van de permeabiliteitscoëfficiënt kon tegengaan na ovariëctomie ($P<0.05$). Dit suggereert dat oestrogeen een beschermend effect heeft op de bloed-hersenbarrière permeabiliteit en dat de toename van deze permeabiliteit tijdens zwangerschap niet wordt veroorzaakt door toegenomen oestrogeenspiegels.

Cerebrale aquaporines (AQPs) spelen mogelijk een rol bij cerebrale oedeemvorming en -resolutie tijdens pathologische condities zoals eclampsie. Daarom werd in **Hoofdstuk 3** met behulp van real-time quantitative polymerase chain reaction gekeken naar de expressie van AQP1, 4 en 9 in rattenhersenen tijdens zwangerschap en de postpartum periode. Hierbij werd expressie van alle drie AQPs gevonden in zowel het anterieure en posterieure cerebrum, het cerebellum alsmede de hersenstam. Dit gold voor zowel de niet-zwangere (n=5) en postpartum (n=5) ratten als ook proefdieren halverwege (n=5) en aan het eind (n=6) van de zwangerschap. Tijdens zwangerschap was de expressie van AQP1 en 9 in het posterieure gedeelte van de hersenen en het cerebellum afgenomen. Ook was er lagere AQP1-expressie in het cerebellum van postpartum versus niet-zwangere ratten. In tegenstelling tot de zwangerschapsgerelateerde afname van de AQP1- en 9-expressie in het posterieure cerebrum en cerebellum, was de AQP4-expressie in deze gebieden toegenomen in zwangere versus niet-zwangere ratten. Echter, AQP4-expressie in het anterieure cerebrum was lager in postpartum dan in niet-zwangere ratten. Omdat de

exacte rol van AQPs in de hersenen nog niet is opgehelderd, zijn de implicaties van deze bevindingen nog speculatief. Mogelijk hebben AQPs invloed op oedeemresolutie en/of de drempel tot het ontstaan van een insult waarbij ook de anatomische locatie een rol speelt.

In **Hoofdstuk 4** is gekeken naar de invloed van pre-eclampsie, naast dat van gezonde zwangerschap, op de myogene activiteit en opbouw van cerebrale weerstandsarteriën. Hiervoor werd gebruik gemaakt van een diermodel voor pre-eclampsie, waarin zwangere ratten behandeld werden met een lage dosis endotoxine. Deze endotoxine veroorzaakt een laaggradige systemische inflammatoire respons die leidt tot symptomen die worden gezien in humane pre-eclampsie, namelijk hypertensie, proteïnurie, gedissemineerde intravasale stolling en gegeneraliseerde endotheelactivatie. Naast cerebrale arteriën werden tevens mesenteriale weerstandsarteriën onderzocht om te bekijken of pre-eclampsie een vergelijkbaar effect had op de perifere vasculatuur.

Niet-zwangere en zwangere ratten werden behandeld met fysiologisch zout (n=9 in beide groepen) of een lage dosis endotoxine (n=9 in de niet-zwangere en n=10 in de zwangere groep). Op dag 20 van de zwangerschap, of 6 dagen na behandeling bij niet-zwangere ratten, werden de myogene activiteit, de druk waarbij geforceerde dilatatie plaatsvond en de structurele eigenschappen onderzocht van de arteria cerebri posterior (ACP) en arteria mesenterica (AM). Het bleek dat zowel gezonde zwangerschap als endotoxine-infusie geen invloed hadden op de structuur en myogene tonus van ACP zolang de drukken binnen de autoregulatoire range bleven. Echter, endotoxinebehandeling leidde bij zwangere ratten tot verlaging van de druk waarop geforceerde dilatatie ontstond vergeleken met gezonde zwangere en niet-zwangere ratten. Dit suggereert dat pre-eclampsie, maar niet de gezonde zwangerschap, predisponeert tot doorbraak van cerebrale autoregulatie en oedeemvorming tijdens bloeddrukstijging zoals bij eclampsie. Ook in AM hadden zwangerschap en endotoxinebehandeling geen effect op de structuur van deze vaten. Echter, in tegenstelling tot in ACP, veroorzaakte zwangerschap een afname van myogene tonus van MA, zonder bijkomende invloed van endotoxine. Dit verschil in bevindingen in myogene tonus tussen ACP en AM kan mogelijk verklaard worden door de functionele verschillen tussen het perifere mesenteriale en cerebrale vaatbed. De afname van myogene tonus van AM zou mogelijk een rol kunnen spelen in de afgenomen perifere vasculaire weerstand die tijdens zwangerschap optreedt. De ACP liet geen afname van myogene tonus zien. Dit zou verklaard kunnen worden door het feit dat in het cerebrale vaatbed strikte autoregulatie van bloeddoorstroming nodig is om ischemie of hyperperfusie te voorkomen. Mogelijk is dit tijdens zwangerschap des te meer van belang, gezien het toegenomen plasmavolume en hartminuutvolume.

Om te onderzoeken of bovengenoemde veranderingen in ACP-functie waren gerelateerd aan andere vormen van schade in de hersenen, is in **Hoofdstuk 5** gekeken naar de aanwezigheid van hersenschade in met endotoxine behandelde zwangere ratten, een

model voor pre-eclampsie. In dit onderzoek werd de aanwezigheid van S100B, een marker voor neuroinflammatie, gemeten in de hersenen en de perifere circulatie. Hierbij werden geen verschillen in cerebrale S100B-expressie gevonden tussen niet-zwangere ratten behandeld met fysiologisch zout (n=5) of endotoxine (n=7) en zwangere ratten behandeld met fysiologisch zout (n=7) of endotoxine (n=7). Ook plasmaspiegels van S100B waren vergelijkbaar bij zwangere ratten die waren behandeld met endotoxine of fysiologisch zout. Uit deze resultaten concludeerden we dat zwangerschap en experimentele pre-eclampsie de cerebrale expressie en plasmaspiegels van S100B niet beïnvloeden. Dit suggereert dat er geen sprake was van betrokkenheid van de hersenen in ratten met experimentele pre-eclampsie.

Deel II - Humane studies

In **Hoofdstuk 6** wordt een overzicht gegeven van de bestaande literatuur over visusstoornissen bij patiënten met (pre-)eclampsie en HELLP-syndroom. Aangezien visusstoornissen relatief vaak voorkomen bij (pre-)eclampsie patiënten, kunnen obstetrici regelmatig geconfronteerd worden met pathologie van het visuele systeem. Naast dat de meeste symptomen onverklaard blijven en van voorbijgaande aard zijn, zijn verschillende oogheelkundige afwijkingen nadrukkelijk geassocieerd met (pre-)eclampsie, waaronder corticale blindheid, sereuze retinaloslating, retinopathie van Purtscher, veneuze stamocclusie en bloedingen in de retina of het glasvocht. Visuele symptomen die als gevolg van deze afwijkingen door patiënten kunnen worden geuit zijn wazig zien, amaurosis fugax, fotopsie en scotomen, waaronder homonieme hemianopsie. Over het algemeen is, behalve het verlagen van de bloeddruk en voorkomen van insulten, geen specifieke behandeling geïndiceerd voor patiënten met pre-eclampsie en visusstoornissen. In het merendeel van de gevallen herstelt de gezichtsscherpte zich na de zwangerschap. Permanente visusstoornissen na (pre-)eclampsie zijn echter incidenteel beschreven en zijn gerelateerd aan persisterende cerebrale laesies, infarcering, en/of afwijkingen van de retina.

Jaren na het doormaken van (pre-)eclampsie zijn bij deze vrouwen cerebrale wittestoflaesies beschreven. Mogelijk zijn deze laesies gerelateerd aan visusproblemen, voornamelijk wanneer ze gelegen zijn in de cerebrale visuele gebieden. Daarom was het doel van **Hoofdstuk 7** om te onderzoeken of voormalig eclamptische vrouwen een lagere visusgerelateerde kwaliteit van leven ervaren. Voormalig eclamptische vrouwen (n=46) en controles die (een) normotensieve zwangerschap(pen) hadden doorgemaakt (n=47) vulden een gevalideerde visus-gerelateerde kwaliteit van leven vragenlijst (NEI-VFQ-39/NL) in. Samengestelde scores en 4 van de 12 subschaalscores van de NEI-VFQ-39/NL, te weten algemene gezondheid, visusgerelateerd sociaal functioneren, autorijden en perifere zien, waren significant lager voor voormalig eclamptische vrouwen vergeleken met controles ($P<0.01$ voor samengestelde scores). Om te bekijken of deze lagere

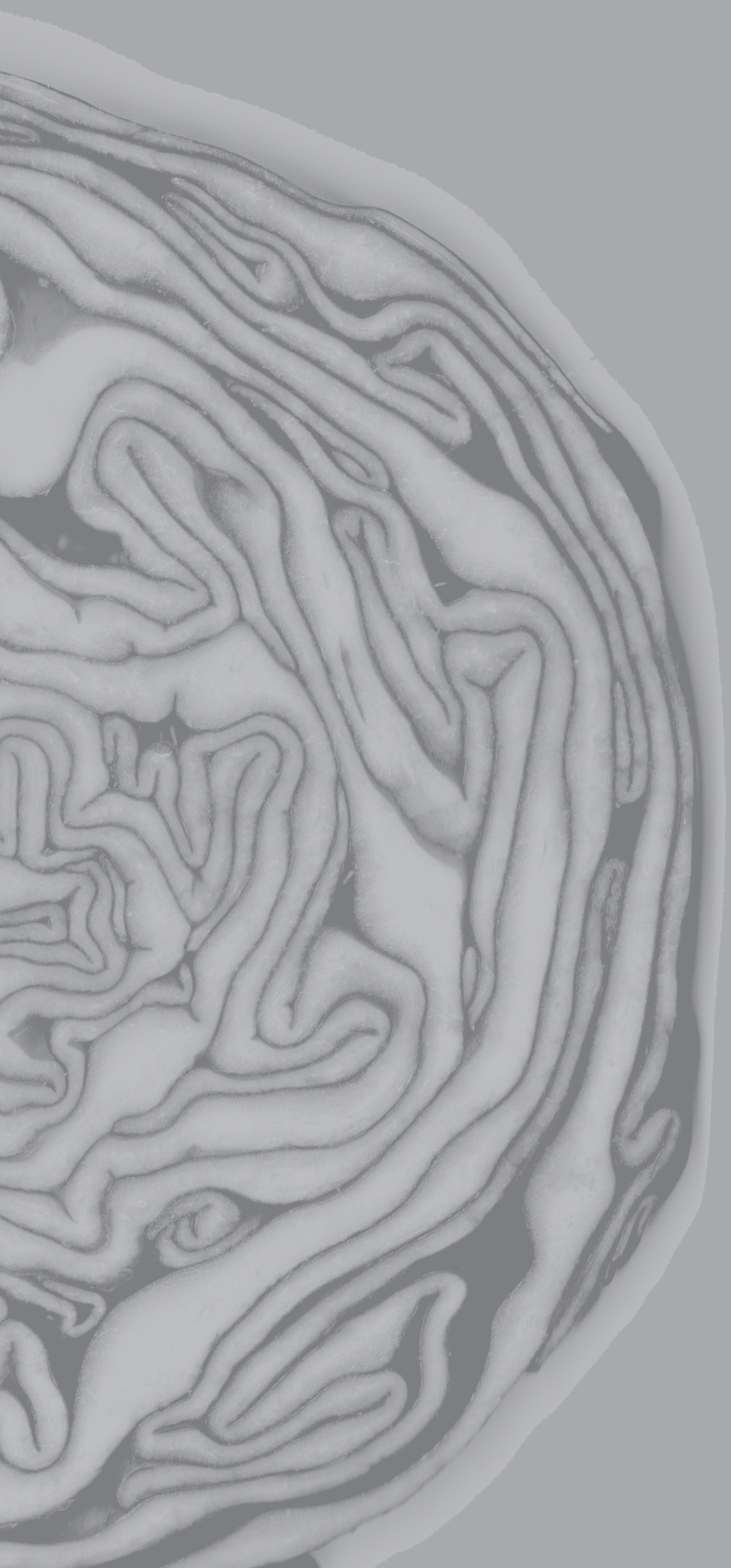
scores van voormalig eclamptische vrouwen verklaard zouden kunnen worden door gezichtsvelduitval als gevolg van wittestoflaesies, werd bij hen een gezichtsveldonderzoek verricht (n=43). De gezichtsvelden van alle vrouwen bleken intact, wat suggereert dat de lagere visusgerelateerde kwaliteit van leven niet was geassocieerd met gezichtsvelduitval. Echter, bij 35,7% van de voormalig eclamptische vrouwen die een MRI ondergingen (n=42) waren wittestoflaesies aanwezig, en deze laesies waren geassocieerd met lagere NEI-VFQ-39/NL samengestelde scores en 4 van de 12 subschaalscores, te weten algemeen gezichtsvermogen, dichtbij zien, visusgerelateerde rolproblemen en perifere zien ($P<0.05$ voor samengestelde scores). Deze lagere scores in de subgroep van voormalig eclamptische vrouwen met wittestoflaesies zouden gerelateerd kunnen zijn aan verminderd functioneren van hogere orde visuele functies als gevolg van wittestoflaesies in de cerebrale gebieden die bij deze functies betrokken zijn.

Hoofdstuk 8 had tot doel om inzicht te krijgen in de mogelijke pathogenese en consequenties van cerebrale wittestoflaesies na eclampsie, die werden beschreven in het vorige hoofdstuk. Hiervoor werd gekeken naar de regionale verdeling van deze laesies op MRI-scans van vrouwen jaren na het doormaken van eclampsie (n=63), pre-eclampsie (n=74) en normotensieve zwangerschappen (n=75). Dit liet, in overeenstemming met eerder onderzoek, zien dat voormalig (pre-)eclamptische vrouwen vaker wittestoflaesies hadden dan vrouwen die normotensieve zwangerschappen hadden gehad (35.0% versus 21.3%; $P<0.05$). Bovendien waren deze laesies ernstiger in de (pre-)eclampsie groep vergeleken met de groep met normotensieve zwangerschappen (0.07 versus 0.02 mL; $P<0.05$). In alle groepen was het merendeel van de wittestoflaesies gelegen in de frontale kwab, gevolgd door de pariëtale, insulaire en temporale kwabben. Hieruit werd geconcludeerd dat de regionale verdeling van wittestoflaesies vergelijkbaar was voor alle drie groepen. Opvallend is dat deze verdeling overeenkomt met de verdeling van wittestoflaesies bij condities die geassocieerd zijn met cerebro-/cardiovasculaire ziekte, zoals migraine en dementie. De wittestoflaesies bij voormalig (pre-)eclamptische vrouwen zouden daarom mogelijk een uiting kunnen zijn van de predispositie van deze vrouwen voor cerebro-/cardiovaculaire ziekte. Deze bevindingen benadrukken, naast de reeds bestaande literatuur, het belang van evaluatie van cerebro-/cardiovaculaire risicofactoren bij voormalig (pre-)eclamptische vrouwen. Daarnaast is gesuggereerd dat het doormaken van 'posterior reversible encephalopathy syndrome' (PRES), het onderliggende mechanisme achter de neurologische symptomen in de acute fase van (pre-)eclampsie, mogelijk ook een rol speelt bij het ontstaan van wittestoflaesies. Gezien de regionale verdeling van wittestoflaesies, veel meer frontaal dan posterieur, lijkt dit echter minder waarschijnlijk.

Tot slot worden in **Hoofdstuk 9** een algemene discussie, conclusies en mogelijkheden voor toekomstig onderzoek gegeven. Uit de dierstudies wordt geconcludeerd dat zwangerschap, en pre-eclampsie in het bijzonder, verschillende facetten van de hersenen

en cerebrale circulatie beïnvloedt en dat dit mogelijk leidt tot een toegenomen vatbaarheid van de hersenen voor ontwikkeling van oedeem tijdens acute bloeddrukstijging. Zowel de tijdens zwangerschap gevonden toegenomen permeabiliteitscoëfficient van cerebrale vaten voor Lucifer Yellow als de veranderde aquaporine-expressie kunnen invloed hebben op cerebrale oedeemvorming en/of –resolutie. De exacte implicaties van deze zwangerschapsgerelateerde cerebrale adaptatie zouden in toekomstig onderzoek verder moeten worden onderzocht. Naast de invloed van gezonde zwangerschap, lijkt pre-eclampsie de kwetsbaarheid voor cerebrale oedeemvorming verder te doen toenemen. Pre-eclampsie leidde in hersenvaten namelijk tot geforceerde dilatatie bij lagere drukken dan in gezonde zwangerschap. Dit suggereert een toegenomen kwetsbaarheid voor bloed-hersenbarrièredoorbraak en vervolgens oedeemvorming. Deze kwetsbaarheid lijkt niet geassocieerd te zijn met andere vormen van hersenbetrokkenheid, zoals werd onderzocht met de neuroinflammatoire marker S100B.

Met betrekking tot de klinische studies wordt in aanvulling op eerder onderzoek geconcludeerd dat (pre-)eclampsie niet langer als een compleet reversibele aandoening kan worden beschouwd waarvan vrouwen geheel genezen. Jaren na het doormaken van eclampsie hebben vrouwen namelijk een lagere visusgerelateerde kwaliteit van leven dan vrouwen die een normotensieve zwangerschap doormaakten. Hierbij lijkt er een relatie te zijn met aanwezigheid van cerebrale wittestoflaesies, die vaker voorkomen na (pre-)eclampsie dan na een normotensieve zwangerschap. Voorts suggereert de regionale verdeling van deze wittestoflaesies een verband met een constitutionele aanleg voor cerebro-/cardiovasculaire ziekte die voormalig (pre-)eclamptische vrouwen hebben. Hiermee wordt het belang van evaluatie en reductie van cerebro-/cardiovasculaire risicofactoren bij deze vrouwen nogmaals benadrukt.



DANKWOORD

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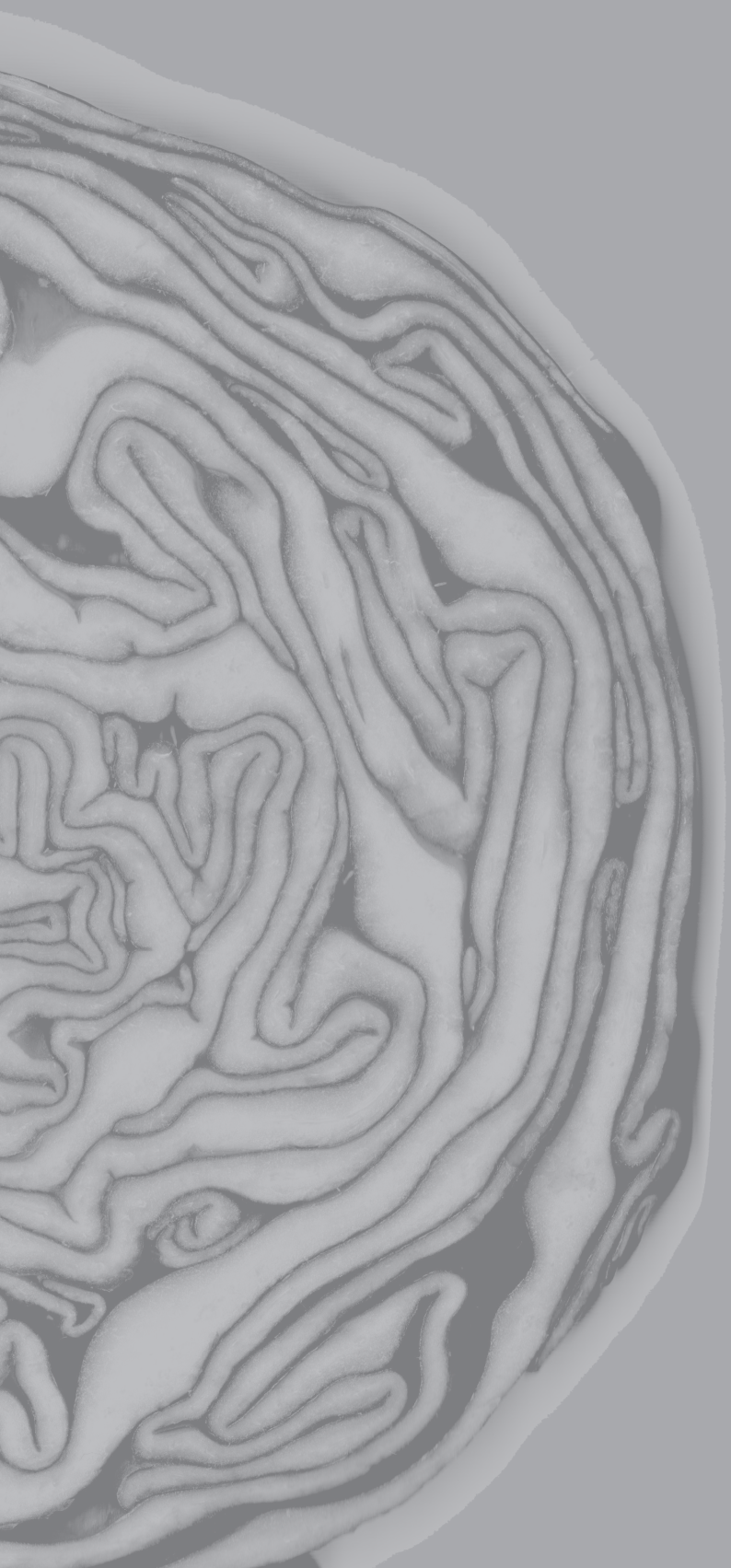
Marjon



CURRICULUM VITAE

Marjon Wiegman werd geboren op 10 mei 1985 te Groningen. Zij bezocht het Praedinius Gymnasium in deze stad en behaalde daar in 2003 cum laude haar eindexamen. Aansluitend startte zij haar studie geneeskunde aan de Rijksuniversiteit Groningen. Tijdens haar 3e studiejaar kwam zij in contact met dr. G.G. Zeeman, door wie zij enthousiast raakte voor het doen van onderzoek binnen de afdeling Obstetrie van het Universitair Medisch Centrum Groningen (UMCG). Dit resulteerde onder andere in een verblijf in Vermont (VS), waar zij in 2007 gedurende een jaar onderzoek deed naar de cerebrale circulatie tijdens zwangerschap bij de onderzoeksgroep van prof.dr. M.J. Cipolla. Vervolgens werd zij toegelaten tot het MD/PhD-traject van de Junior Scientific Masterclass om onder leiding van prof.dr. J.G. Aarnoudse, prof.dr. M.J. Cipolla, dr. M.M. Faas en dr. G.G. Zeeman promotieonderzoek te doen naar de pathofysiologie en langetermijngevolgen van eclampsie. Marjon heeft haar co-schappen achtereenvolgens gelopen in het UMCG en de Tjongerschans te Heerenveen. Haar semi-arts stage heeft zij gedaan bij de afdeling Dermatologie van het UMCG en de afdeling Pathologie van de Isala Klinieken te Zwolle. In de zomer van 2012 behaalde zij cum laude haar artsexamen, waarna zij in september is gestart met de opleiding tot dermatoloog in het UMCG.